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A Review of Nutrition Studies in India (1951-56)

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PREFACE

A review of nutrition researches in India was published in 1951 by the Indian Council of Medical Research. It appeared in non-technical language. A separate, extensive, bibliography was included for the convenience of the scientists.

Activity in the field of nutrition in India had increased considerably since the publication of the review in 1951 and it was felt that a brief account of researches during the five year period 1951-56 was called for. The present publication, prepared on a suggestion from the Nutrition Advisory Committee, is principally intended for scientific workers. It is not a critical review in the strictest sense of the term. The authors have given as wide a coverage as has been possible, as will be evident from the list of references given at the end of each chapter. It is hoped that this review will meet the need of those scientists who are interested in nutrition and who would prefer to have all the relevant information within the covers of a single publication.

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FOODSTUFFS

Cereals

Rice.

The production of paddy in India accounts for 21 per cent of the world output, although 32 per cent of the total area in the world under rice cultivation is in India. This is probably due to low yields (900 lb./acre) obtained by the indigenous method of rice cultivation as compared to Japan's 3,200 lb. and China's 2,200 lb./acre (Parthasarthy, 1953). Owing chiefly to Government initiative, more and more cultivable area is being brought under the Japanese method of cultivation which is known to give high yields. Experimental farms in India have grown 2,000-4,000 lb./acre. We would be advancing considerably towards attainment of self-sufficiency in foodstuffs by improving the techniques of cultivation.

Coloured and coarser varieties are reported to be richer in protein, minerals and vitamins. Since parboiled rice is usually made from coarse varieties, the resultant product is also richer in nutrients, particularly as losses on milling are insignificant. Washing and cooking losses are of the order of 4-10 per cent for protein, 15-30 per cent for minerals and 30 per cent or more for B vitamins (Sreenivasan, 1951).

Paddy parboiled in calcium chloride solution was found to produce rice with higher calcium content than rice parboiled in tap water (Narayana Rao and Swaminathan, 1954).

The Food and Agricultural Organisation Nutrition Committee for South East Asia has recommended that rice should not be milled to such an extent as to make its vitamin B_1 content less than 1·8 μg./g. Rats fed on diets containing rice milled according to this specification, showed a higher growth rate than on raw milled rice of low-extraction rate containing 1·1 μg./g. of vitamin B_1/g. (Narayana Rao and Swaminathan, 1953). During the earlier stages of milling more phytate P is lost than vitamin B_1, but when 13 to 17 per cent millings are removed losses in vitamin B_1 and phytate P are almost equal—more than 70 per cent each (Sathe *et al.*, 1951). The essential amino acid (E.A.A.) content estimated microbiologically in several varieties of cereals have been reported (Balasubramanian *et al.*, 1952).

Wheat.

A high-yielding crop of wheat had slightly higher protein and calcium and a little less phosphorous than an average-yielding wheat crop. However, both samples had identical contents of moisture, fat, gluten, fibre, carbohydrate and vitamin B_1. Also the protein efficiency ratio (P.E.R.) of the two samples were almost the same (Esh and Basu, 1952). Nitrogenous fertilisers in combination with super-phosphates are reported to have yielded a crop with higher protein content, compared to super- phosphates alone (Bains, 1953).

Millets.

Millets and subsidiary cereals form an important constituent in the diets of a substantial proportion of the population in India, especially of the lower economic groups. The nutritive value of foodstuffs such as *Paspalum scrobiculatum* ('Kodra') *Amaranthus paniculatus* ('Rajgira') and *Panicum miliaceum* ('Wari') *Sorghum vulgare* ('Red milo' or 'jowar') and *Setaria italica* ('Italian millet') has been evaluated (Kundaji and Rao, 1954). Generally they were inferior to wheat in protein, minerals and vitamin B_1 ; their proteins had a lower nutritive value than wheat proteins (Kundaji and Rao, *loc. cit.* ; Kadkol, Srinivasamurthy and Swaminathan, 1954 ; Kadkol and Swaminathan, 1955 ; Sivaramakrishnan and Sarma, 1951 ; and Sur, Swaminathan and Subramanyan, 1955).

The seeds of *Amaranthus paniculatus Linn* were found on analysis to contain crude protein as high as 14·5 to 16 per cent and the ash which formed 3·6 per cent, contained calcium to the extent of 6 per cent. Determinations of the biological value and digestibility coefficient of the protein of the raw seed showed it to be of good nutritive value ; the protein efficiency ratio at 10 per cent level, was found to be comparable to that of casein (Subramanian and Srinivasan, 1951).

Bamboo seeds with a crude protein content of 12 per cent, were comparable with wheat and superior to rice ; the biological value of the bamboo seed proteins was found to be as high as that of rice proteins and higher than that of wheat proteins. The complete replacement of rice in a poor rice diet by the seeds enhanced growth in rats by about 50 per cent (Lakshminarayan *et al.*, 1955).

Tapioca.

Tapioca is a staple food of the poor on the West Coast of South India, particularly of Kerala. In recent years much work has been done on the role of supplementary foodstuffs in the tapioca diet.

Suitable Indian foods could be prepared by gelatinising tapioca flour partly, using hot water and incorporating cereal and/or legume flours in amounts from 5 to 20 per cent (Bains, 1953). Experiments on six healthy adult males showed that on rice and rice and tapioca diets N balances were similar; Ca and P balances were better on a combination of rice-tapioca diet than on rice diet alone (Murthy *et al.*, 1954). It was also reported that children's health as observed for six months did not suffer from the substitution of tapioca for 25 per cent of the rice in their diet (Reddy *et al.*, 1954).

Animals fed on tapioca as the only source of protein could not survive beyond five weeks. The minimum proportion of rice to tapioca capable of maintaining life is 30-40 only at this proportion. N equilibrium is just maintained in rats (Rao *et al.*, 1951).

In a poor rice diet, even 50 per cent replacement of rice by tapioca, has no deleterious effect on growth of albino rats. This is attributed mainly to the calcium in tapioca (Subrahmanyan *et al.*, 1954).

A synthetic product with an approximate composition of 20 per cent wheat flour, 15 per cent groundnut flour and 60 per cent tapioca has been prepared. Attempts have been made to make it look like rice by suitable processing. Its chemical composition is 9·5 per cent moisture, 14·2 per cent protein, 1·9 per cent fat, CHO 71·7 per cent, Ca 0·19 per cent and P 0·15 per cent vitamin B_1 0·22 mg. per cent and calories 361/100 gm. (Subrahmanyan, 1952 ; and Subrahmanyan *et al.*, 1954).

PULSES

Nutritive value and its improvement.

Different pulses have been analysed for their proximate composition, vitamin and essential amino acid (E.A.A.) contents. The alleged 'toxicity' of pulses has been found to be due to the deficiency of calcium and phosphorus and not to any amino acid imbalance or the absence of any unidentified growth factor in such a diet (De, 1953). The limiting amino acids in pulses have been found to be methionine, cystine and tryptophan (Vijayaraghavan *et al.*, 1953). Methionine content of different cereals and legumes has been estimated and the low nutritive value of protein from lentil and *khesari dhal* (*L. sativus*) is attributed to their low methionine content (Rudra and Chowdhry, 1950). Pulses have been reported to have a higher strepogenin activity, chiefly residing in the globulin fraction, than even casein (Murthy and De, 1951).

Considerable work has been done on the possible improvement of the nutritive value of different pulses by suitable processing. Germination of pulses up to 4 days has been found to improve their nutrient contents generally. Germination improved the biological value of pulses, while not having any effect on the trypsin inhibitor activity (Chattopadhyay and Banerjee, 1953). Increases in biotin, niacin, pyridoxine, pantothenic acid, inositol, tocopherol and vitamin K values and a reduction in folic acid have been observed after germination (Banerjee *et al.*, 1954 ; Banerji *et al.*, 1955 ; and Chattopadhyay and Banerjee, 1952). During germination of seeds of mung, masoor and black pea for 24 hours after overnight soaking, 7 and 14 per cent of the total ascorbic acid formed leaches out, further loss was negligible up to 8 days (Sreenivasan and Wandrekar, 1951). The alleged vitamin B_{12} activity in germinated and un-germinated Indian pulses has been found to be attributable to alkali-stable desoxyribosides, the increase in the activity on germination being due to increase in the nuclease activity (Rohatgi *et al.*, 1955 ; Ramachandran and Phansalkar, 1956 ; and Sreenivasan and Lavate, 1956). During germination of pulses phytin P diminished (Belavady and Banerjee, 1953), whereas the availability of iron showed an increase (Singh and Banerjee, 1955).

The nutritive value of Bengal gram (*Cicer arietinum*) Horse gram (*Dolichose biflorus*) and field bean (*Dolichos lablab*) could be improved by autoclaving at 15 lb. pressure for 15 minutes, which process destroyed the trypsin inhibitor. Some pulses like red gram (*Cajanus indicus*) and field beans could be improved in their nutritive value by methionine supplementation (Hirwe and Magar, 1951 and 1953).

Appreciable differences in the (E.A.A.) contents of the different strains of the same pulses and cereals has been reported. It has been emphasised that heredity and environmental factors influence the (E.A.A.) and vitamin contents and thus the nutritive value (Chitre, Desai and Bhavani, 1948 ; Chitre, Desai and Raut, 1955 ; and Chitre *et al.*, 1956).

VEGETABLES

The nutritive value of various vegetables has been determined. Leafy vegetables contained large amounts of riboflavin as also condiments, green peas and papaya; roots and tubers were poor sources of the vitamin (Ghosh and Rajagopal, 1951). Leafy vegetables are a very good source of calcium which was utilised by experimental animals as well as from skim milk (Devadatta and Appanna, 1954). In parts of South India, the lichen *Parmelia tinctorum* which is commonly used in various food

preparations has a Ca and Fe content comparable to that of green leafy vegetables (Lal and Rao, 1956). Among the non-leafy vegetables *Agave* as the sole source of carbohydrates in synthetic diet did not support growth in rats, but added as a non-leafy vegetable to a rice diet, it produced better growth than Knol-khol which it replaced. The value of *Agave* was due mainly to its high Ca content (Subrahmanyan, *et al.*, 1953). The E.A.A. content of amaranth (*Amaranthus gangeticus* and aconite bean (*Phaseolus aconitifolius*) and amaranth and *Trigonella faenum-graecum* and that of pumpkin protein has been determined (Deshpande *et al.*, 1954 ; and Kamath and Sohonie, 1955 and 1956). *Amaranth* was found to be superior to *aconite* bean in supplementing poor rice diet for growth in young rats (Lal and Rao, *loc. cit.*). Lucerne, Dub grass and drumstick have high supplementary values to a poor rice diet (Sur, 1955). In cooking of vegetables exposed to air, loss of vitamins was 5 to 12 per cent and in pressure-cooking, 8 to 29 per cent.

OTHER FOODSTUFFS

Some fruits have been analysed for their acid and sugar contents (Siddappa and Bhatia, 1952 ; Lewis *et al.*, 1954 ; Lulla and Johar, 1954 ; and Bhatia *et al.*, 1955). Several species of fish have been analysed for their proximate principles (Vekataraman and Chari, 1951 ; Saha and Chowdhury 1951 ; Saha *et al.*, 1951 ; Venkataraman and Chari, 1951 and Joshi and Magar, 1955) and also for their E.A.A. content (Master and Magar, 1954). Coconut meal was found to have a good supplementary value to a poor rice or tapioca diet (Sadasivan, 1950 and 1951). Iron in palm and cane jaggeries has been found to be easily available for haemoglobin regeneration in rats rendered anaemic by bleeding (Joshi and Sohonie, 1956). It was reported that the percentage of sulphur retained was greatest from autoclaved soyabeans and least from raw soyabeans ; from germinated beans it was more than from raw and less than from autoclaved beans ; soya milk was only slightly better than raw beans. The excretion of cystine S was least on the raw soyabean diet, higher on the autoclaved soyabean and still higher on the germinated soyabean and soya milk diets. Excretion of methionine S was highest on the raw soyabean, lower on the soya milk and germinated soyabean diets and lowest on the autoclaved soyabean diet (Viswanathan and De, 1951).

MILK

Determinations of different constituents of milk of different species and different breeds of the same species during different stages of lactation have been reported by several workers (Table).

TABLE.

	Cow	Buffalo	Goat	Sheep	Ref.
1. F.P. Depression	−0·548°C	−0·552°C	−0·579°C	−0·587°C	Dharmarajan *et al.*, 1950 ; Dastur *et al.*, 1952 ; and Dharmarajan *et al.*, 1954.
2. Lactose per cent	4·92	5·26	4·45	..	
3. Chloride per cent	0·089	0·072	0·127	..	
4. Lactic acid per cent	0·12	0·12	0·14	0·17	
5. Total N mg./100 ml.	526·2	..	..	..	
6. N.P.N.	25·82	..	Nearly double the amt. of urea N compd. to cow milk	Rich in N.P.N., amino aicd N.	
7. Urea N mg./100 ml.	11·60	..	..	..	Venkatappaiah *et al.*, 1952.
8. Ammonia N ,,	0·26	..	..	..	
9. Uric acid N ,,	0·54	..	..	..	
10. Creatinine ,,	0·62	Higher creatinine			
11. Amino acid N ,,	4·04	..	..	..	
12. Undetermined NPN ,,	8·32	..	..	..	
13. Phospholipids ,,	31·37	21·03	49·69	58·51	Baliga, and Basu, 1956.
14. Fat content per cent	4·4	6·1	5·1	8·1	
15. Cholesterol mg./100 ml.	No diff. due to breeds and species 1-8mg./100ml.				Gulvady *et al.*, 1952.
16. Tocopherol r/lb. milk.	837	896	..	..	Narayanan *et al.*, 1956.
17. Carotene r/g. butter fat	4·8	..	..	..	
18. Vitamin A.I.U./lb.	620	906	..	..	
19. Vitamin B_1 r/ml.	0·54	0·50	0·97	..	Venkateswara Rao and Basu, 1951.
20. Riboflavin r/ml.	1·32	1·07	0·75	1·85	
21. Nicotinic acid r/ml.	0·83	0·83	1·30	1·42	

The N.P.N. of milk of cows, buffaloes, goats and sheep have been studied under identical feeding conditions. The concentration of the N.P.N. in the milk of different breeds of cow did not vary. Sheep milk was rich in N.P.N. and amino acid N. Goat milk contained nearly double the amount of urea N as compared with cow milk. The uric acid content of cow milk was much higher than that of the milk of other species of animals. The creatinine content of buffalo and sheep milk

was relatively high. The season of the year and the stage of lactation were found to have no influence on the concentration of the N.P.N. of milk (Venkatappaiah and Basu, 1952).

On prolonged boiling of milk, urea N and uric acid decreased and ammonia N increased significantly (Venkatappaiah and Basu, 1955). The E.A.A. composition determined microbiologically in the milk of Indian buffaloes were found to be practically similar to that reported for cow's milk (Joshi and Raj, 1954 and 1955).

The cholesterol content varied from 1-5 and 1-8 mg./100 ml. in the milk of individual cows and buffaloes respectively. Higher values were found in milk towards the end of lactation. The season of the year had no effect on the concentration of cholesterol in milk (Gulvady *et al., loc. cit.*).

Both fat soluble and water miscible forms of synthetic vitamin A when added to milk were stable to pasteurisation and boiling (Shroff *et al.*, 1954) percentage fat and vitamin A potency of the milk remained unaltered when cows on a diet of high carotene content were given shark-liver oil supplement. On a diet of medium carotene content and in the dry fodder period a supplement of 1,00,000 to 2,00,000 I.U. of vitamin A caused an increase in the vitamin A potency of the milk fat from cows and buffaloes (Narayanan *et al.*, 1953)

MILK PRODUCTS

Milk is consumed in India not only as such, but also in other forms like *dahi, khoa, kheer* and *channa*. Analyses of boiled milk and these milk products have been carried out to evaluate their comparative nutritive value. *Dahi* had lower vitamin A, vitamin B riboflavin and nicotinic acid as compared to milk ; *khoa* and *kheer* had lower vitamin A and vitamin C than milk and *channa* was the poorest especially in vitamins A, B complex and C (Mani *et al.*, 1955). Feeding experiments with rats for three successive generations exclusively on milk or milk products alone showed milk to be the best for promoting growth followed by *kheer, dahi, khoa* and *channa,* in this order. The reproductive and lactating performances of the animals observed over three generations ranged in the order of milk, *dahi, kheer* and *khoa*. Rats on *channa* proved sterile even in the first generation (Lily *et al.*, 1955). Milk or milk products showed good supplementary effect to poor rice diet when fed to albino rats (Lily *et al., loc. cit.*).

Dahi or curds.

After souring there was a marked fall in specific gravity and pH. Lactose and total solids decreased. There was an increase in the

amounts of lactic and volatile acids, ethanol and dialysable Ca and P and N.P.N. Protein N decreased. A rapid increase in ammonia N was also found to occur during souring, but it often decreased in the *dahi* when stored for several days (Khanbatta and Dastur, 1950 and 1951).

Curd feeding brought about an increase in the coliform organisms in the faeces and also the biosynthesis of vitamin B_1 in the intestines. The action of sulphaguanidine in greatly reducing the excretion of vitamin B_1 and suppressing the growth of coliform organisms were prevented by curds. The action of curds in promoting intestinal biosynthesis of vitamin B_1, has been confirmed in a group of children. Curd was found to help in the attainment of a better thiamine nutritional status through the intestinal synthesis of the vitamin (Balakrishnan and Rajagopalan, 1953, 1954 and 1955).

Ghee.

The effect of the method of preparation of ghee, on its keeping quality has been studied. Peroxides developed more slowly in the samples prepared at a low temperature (40°C.) than in those where higher melting temperatures had been used. Ghee prepared by the indigenous method developed peroxides faster than creamery ghee (Balakrishnan and Rajagopalan, 1955).

Considerable loss of vitamin A occurred at the butter stage in the preparation of ghee. Butter prepared from fresh milk lost after 6 days, 12 to 20 per cent of its vitamin A. Part of this loss could be prevented by using boiled milk and by washing the butter before storage. Butter stored under water or in a 2 per cent salt solution took about 5 times as long to lose this proportion of vitamin A.

Whole milk fortified with oil soluble vitamin A and skim milk fortified with water miscible vitamin A retained their vitamin A after souring for 24 hours. Vitamin A was almost unaffected when ghee was prepared from cream fortified with water-miscible vitamin by direct cooking at 115°F. (Narayanan *et al.*, 1954). There was much loss of carotene and vitamin A when ghee was stored in mud pots as compared to its storage in glass, aluminium or tin containers. Progressive destruction with time of both vitamin A and carotene from products in metal containers was slow, only 3 per cent of vitamin A being lost in three months and 15 per cent in 6 months, compared to the complete destruction of the vitamin at the end of 3 months in the sample kept in a mud pot (Narayanan *et al.*, 1953).

Vitamin D contents of three samples of South Indian butter, as determined by the line test technique in rats were 1·20, 1·18 and 1·50 I.U. per g. respectively (Ranganathan, 1954).

MILK SUBSTITUTES

The role of milk and milk products in nutrition is well known. But unfortunately in India a considerable proportion of the population cannot afford animal milk. Much work has been done in recent years on milk substitutes of vegetable origin, particularly from groundnut.

It has been found that during the preparation of heated groundnut milk, 16·78 per cent of vitamin B_1, 5·72 per cent of riboflavin and 8·63 per cent of niacin are destroyed. During curd preparation from heated groudnut milk, vitamin B_1 remained unchanged, niacin decreased by 12·8 per cent and riboflavin increased by 21·3 per cent (Moorjani and Bhatia, 1953). During souring, N.P.N. and ammonia N increased, protein N decreased and total N remained unchanged (Moorjani and Bhatia 1954).

Groundnut milk is deficient in calcium and its proteins are of low biological value, hence fortification with calcium and blending it with germinated soyabean in the ratio of 3 : 1 have been attempted to improve its nutritive value. This processed-Ca-fortified groundnut milk when fed to rats with the poor rice diet, improved their growth rate significantly (Moorjani and Subrahmanyan, 1950). The growth promoting activity of protein in this product reached an efficiency up to 80 per cent of that shown by whole milk protein and calcium utilisation was equal to that in the reconstituted whole milk powder (Moorjani *et al.*, 1955).

The efficiency of protein of the vegetable milk for haemoglobin regeneration, r.b.c. formation, and maintenance of total serum-protein and albumin levels varied between 70 and 92 per cent of that possessed by casein (Moorjani and Subrahmanyan, 1950).

Supplementation of a rice and vegetable diet with 1 oz. daily of pressed groundnut cake flour, skimmed milk powder or 12 oz. of fortified groundnut milk was found to be beneficial in increasing the height, and weight of young boys and girls. The general health of the children was also found to have improved with either of the supplements (Lal, 1952 ; and Subrahmanyan *et al.*, 1954).

PRESERVATION OF FOODSTUFFS

The effects of storage of foodstuffs as such, and after processing and canning, have been widely studied. Tapioca flour, groundnut flour and their mixture in a 4 : 1 ratio were stored in gunny bags at room temperature or at 37°C. for 5 months. Negligible changes were found in the tapioca flour. The free fatty acids and peroxide values of fat in the groundnut flour and its mixture with tapioca, increased during storage. The nutritive value of the flour mixture and the biological value of the groundnut protein did not decrease on storage (Subrahmanyan *et al.*, 1954). When grains infested with insects were stored, weight loss was considerable. There was no effect on the total N or sugar contents. The rise in acidity of fat was greater in infested samples (Pingle *et al.*, 1954). There was a change in the proportions of free amino acids when rice samples were stored for 1 to 3 years though the amino acid pattern was similar in all the samples. The proportions of alanine, threonine and glutamic acid and the amounts of lysine and histidine decreased with age. In parboiled rice, tryptophan, methionine, valine, alanine, threonine and glutamic acid were comparable with the amounts found in raw rice stored for the same period. Cystine was present in some of the old samples, but not in the fresh one (Parihar, 1954).

New raw rice contained only fructose, glucose and sucrose in the sugar component. All the old samples contained in addition galactose, maltose, raffinose and maltotriose. Isomaltose and maltotetraose were found only in 3 year old samples. The changes in old samples, possibly due mostly to the action of α amylase on starch, may improve digestibility and taste (Parihar, 1954 and 1955). The high amylase activity of freshly harvested rice decreased rapidly on storage ; cooking quality as measured by the swelling capacity improved. All samples showed (in 1 year) 20 to 30 per cent loss of vitamin B_1 except the parboiled undermilled rice, which showed a considerably lower loss. Total N and digestibility of protein remained unchanged, but N soluble in 3 per cent NaCl decreased, indicating denaturation (Narayana Rao *et al.*, 1954). There was considerable loss of phytin P when infested rice grains were stored (Ghosh, 1952).

Rice stored in sealed amber bottles for 6 months, showed no change in thiamine content, whereas riboflavin increased. Rice stored in gunny bags resulted in a considerable loss of thiamine and a larger gain in riboflavin ; with either forms of storage nicotinic acid content was unchanged. In other cereals thiamine and nicotinic acid did not

alter ; their riboflavin content rose to the same extent in bottles or in gunny bags. Pulses showed no change in thiamine or nicotinic acid but there was increase in riboflavin only when stored in gunny bags (Chitre *et al.*, 1955).

During the canning of vegetables, vitamin C is the most affected, the major portion being destroyed in the blanching process itself. About 83 per cent of the vitamin C in canned pineapple was lost during storage for 4 months at the room temperature of 28-30°C ; Canned potatoes lost 67 per cent after 4 months and canned guavas 52 per cent. Carotene is the least affected during processing. Processed fruits retain more of the vitamins than processed vegetables (Dhopeshwarkar and Magar, 1950, 1952, 1954 and 1955 ; and Malakar *et al.*, 1955).

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CARBOHYDRATES

Subrahmanyan *et al.* (1956) analysed the tender kernels of palmyra palm which are relished in our country for their thirst quenching properties ; they contained besides 92·7 per cent moisture, sucrose 0·38 per cent, fructose 1·46 per cent and glucose 3·21 per cent. The polysaccharide yielded D-galactose and D-mannose.

Bhattacharya *et al.* (1951 and 1951*a*) have studied the comparative metabolism of glucose and fructose in rabbits. In experimental hypoglycemia induced by insulin, intravenous injection of glucose raised the blood sugar a little above the normal within one hour, whereas injections of invert sugar and fructose raised the blood sugar to a level well below the normal. They also found that the liver glycogen in rabbits rose from the fasting average of 1·09 per cent to 2·5 per cent after glucose, 3·65 per cent after fructose and 3·33 per cent after invert sugar, all sugars having been given at a dosage of 2·5 g./kg. body weight intravenously 90 minutes before killing the animals.

The role of fat on utilization of lactose in milk by rats has been investigated by Banerjee (1951) and Roy and Sadhu (1954). In skimmed milk fed group both liver and muscle glycogen and liver neutral fat were significantly lower and liver phospholipid and blood sugar higher than in the whole milk fed group, though the cerebroside contents of the brain of rats on the different diets were the same. The skimmed milk fed rats excreted galactose in the urine which suggested a failure to convert the sugar into glycogen in the liver. No excretion of galactose occurred in rabbits fed skimmed milk. When milk intake and galactosuria produced in rats with skim milk diet reached a constant level, administration of injection enterogastrone, atropine of glucose decreased the galactosuria and acid histamine phosphate or prostigmine increased it.

In different species of mammals, a positive correlation between cerebroside and sphingomyelin and their ratio in brain and lactose in milk was reported by Sadhu (1952). Phospholipid was more variable, but there was a positive correlation between the sphingomyelin : phospholipid ratio in brain and lactose in milk. Sadhu (1953) has also reported an adverse effect of feeding lactose on the levels of cerebrosides and liver sphingomyelin in pigeons.

Nath and Chakrabarti (1951) observed that continued intramuscular injection of an intermediary fat metabolite like sodium β-hydroxybutyrate in rats produced a disturbance in the balance of hepatic glycogenesis-glycogenolysis by causing rapid depletion of liver and muscle glycogen

and increase in liver and muscle lactic acid. Animals treated with "amellin" decreased showed glycogenolysis and less lactic acid in liver and muscle.

Nath and co-workers have studied in detail the role of aceto-acetate in relation to carbohydrate metabolism. Daily injections of increasing amounts of sodium aceto-acetate into normal rabbits caused a rise in the blood lactic acid after 90 days which effect was counteracted when "amellin" also was injected. In this respect "amellin" was found better than insulin. Low daily doses of aceto-acetate over a period of two months produced a drop in the plasma value for ascorbic acid, Nath and Chakrabarti (1951*a*). In scorbutic guinea pigs, aceto-acetate injected intraperitoneally produced a marked increase in blood sugar values and a fall in liver and leg-muscle glycogen ; the change in glycogen was, however, prevented by daily injection of glucose. The animals receiving aceto-acetate had the lowest excretion of vitamin C, Nath *et al.* (1953). Intra-muscular injection of sodium aceto-acetate in rats increased the concentration of ascorbic acid in plasma. In rabbits 60 mg./kg. body weight of aceto-acetate, caused a slight, but temporary increase in plasma ascorbic acid, whereas higher doses had a depressing action, Nath and Chakrabarti (1954). Injections of increasing amounts of aceto-acetate did not affect the concentration of cholesterol in the adrenal glands and blood in normal and scorbutic guinea pigs but caused a lowering of ascorbic acid from the adrenal glands more than in the normals (Nath and Nayudu, 1956).

Basu (1952) reported that in a group of diabetic subjects, given 25 mg. ascorbic acid on alternate days in place of insulin, it was possible to maintain blood sugar at a normal level with half the amount of insulin previously used but the dietary carbohydrate could not be increased. In a second group, where the ascorbic acid was given daily together with insulin, the amount of insulin used could be halved and the dietary carbohydrate could be increased by 100/g. daily while still maintaining the blood sugar at a normal level.

Nath and Sahu (1956) observed that in rats, glucose, followed immediately by aceto-acetate or sodium salt of their condensation product, 2 tetrahydroxybutyl-5-methyl-4-carbethoxyfuran, prevented when injected 45 minutes before alloxan, both diabetes and the decrease of reduced glutathione in the blood. The condensation product was found by Nath and collaborators (Nath and Bhattathiry, 1956; and Nath *et al.*, 1956) to prevent alloxan diabetes in rabbits when injected 5 minutes before alloxan or 2 minutes before the injection of dehydro-ascorbic acid. Blood urea and plasma alkaline phosphatase which increase in alloxan diabetes, remained normal. It was, however, without effect when injected 10 minutes after the dehydro-ascorbic acid.

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FATS

Changes in fat on storage :

Mukherjee (1951) has discussed the probable mechanism of rancidifaction by the action of micro-organisms. Apparently air assisted in the process. First step appeared to be the hydrolysis of glycerides into free fatty acids and glycerol by lipases, followed by rapid oxidation of the individual components by various oxidative enzymes. The author also studied the formation of ketones from fatty acids and on the metabolism of butyric acid by Mukherjee (1952 and 1952*a*). Kantharaj Urs *et al.* (1953) found that the acidity of mustard oil or groundnut oil, crude or refined, increased over 36 weeks when stored in glass or tin containers at room temperature (37°C), whereas it was neutralised to some extent in samples stored in galvanised iron containers by zinc dissolved from the surface of the container. The oils at all temperatures dissolved zinc from the galvanised containers, mustard oil or crude groundnut oil dissolved more zinc than refined groundnut oil and hence it was concluded that the two oils should not be stored in galvanised containers.

Composition :

Application of paper chromatographic technique revealed that cow and buffalo butterfats contain in addition to oleic acids, other octadecenoic acids with double bonds at $\triangle 8$, $\triangle 10$, $\triangle 11$, and $\triangle 12$ (Pathak and Patwardhan, 1953). Similarly the presence in vanaspati (hydrogenated fats) of the $\triangle 8$—, $\triangle 9$—, $\triangle 10$, 11— and 12— octadecenoic acids has been demonstrated (Pathak *et al.*, 1952), and their quantitative estimation carried out by partition chromatography on silica gel (Subbaram and Mahadevan, 1956).

The butter fat from the milk of buffaloes fed cottonseed had higher amounts of oleic, palmitic and stearic acids and lower amounts of lower saturated acids than the values reported in milk fat from buffaloes on a normal diet (Mhaskar and Banerjee, 1954). The component fatty acids have been determined in the fats from the carcass, viscera and liver of some species of Indian fresh water fishes (Pathak and Agarwal, 1952; and Pathak and Pande, 1954). Indian marine fishes, liver of sharks, mature and embryo, the Indian python and the Indian diamond snake Karkhanis and Magar (1955), Indian tiger (Pathak and Agarwal, *loc. cit.*) and turtle (Pathak and Dey, 1956).

Nutritive value :

The nutritive value of various oils and fats has been studied by different workers. Of the different glyceride fractions of buffalo butter fat, obtained by crystallisation from acetone at different temperatures, the fraction obtained at 0°C. was superior to the other fractions as well as the whole fat in inducing growth in rats when fed at 10 per cent level. There was a close association between the iodine value and the growth promoting value of the fractions (Narayana Rao, 1954). The digestibility *in vitro* (estimated with pancreatic lipase) and the nutritive value for rice moth larvae of fat from ten different varieties of 'Bombay fish' were positively correlated, but no correlation existed between digestibility *in vitro*, nutritive value and N : P ratios in various fish fats (Valanju and Sohonie, 1955). The *in vitro* hydrolysis by pancreatic lipase of fresh cow ghee, was inhibited by dissolving in the ghee, the unsaponifiable matter from rancid samples of vegetable oils (Ramamurti and Banerjee, 1950). Ghee of different species were digested *in vitro* more rapidly than the corresponding body fat and the hydrogenated vegetable oils. All ghees were digested at an equal rate irrespective of species. Hydrogenation reduced the digestibility of oils (Bhalerao *et al.*, 1950; Paul *el al.*, 1951).

Different oils and fats fed to rats at 5 per cent level showed little difference in their growth promoting value or in their value for fertility or milk production (Bhalerao *et al.*, 1950*a*). Different oils and fats fed *ad lib* to adult rats with no other food except water, could produce no significant difference in their survival time within the same sex (Venkitasubramanian and De, 1952).

Rats fed a high fat low protein diet for 5 weeks showed pronounced fatty infiltration of the liver when the fat component was of ghee or coconut oil ; rats on mustard or sesame oil had normal liver fat. Inclusion of higher saturated fatty acids at 15 per cent level in a low protein diet resulted in accumulation of liver fat (Damodaran and Sivaraman, 1951).

The nutritive value of vegetable oils was considerably decreased by heating in an open iron pan for a few hours at a high temperature. Fed at 15 per cent level the heated oils group of rats had less gain in weight, less efficiency of feed conversion and had a fatty liver. All rats fed heated oil at 30 per cent level died within one week (Raju and Rajagopalan, 1955).

No significant difference was found in the growth promoting value of safflower seed and niger seed oils when compared to that of groundnut oil or butterfat, even though the former two oils were richer in linoleic

acid glycerides than the latter ones (Narayana Rao and Swaminathan, 1953).

The inclusion of various fats at 10 per cent level in an adequate diet was found to increase the utilization of calcium and phosphorus for rats, as compared with that on a fat-free regimen. The percentage of calcium utilization in rats fed safflower and niger seed oils was "slightly more" [60·1 to 62·0 compared to 55·6 (ghee) to 57·2 (groundnut oil)] than that observed with other fats ; no difference was found in the percentage of N-utilized in diets containing the various fats (Narayana Rao and Swaminathan, 1953*a*).

The digestibility and absorption of blended fats, prepared by mixing straight hardened fat with unhydrogenated groundnut oil were studied in 6 girls 12 to 18 years of age. For blends up to m.p. 40·1°C. digestibility was over 90 per cent and for m.p. above that it fell to 84-87 per cent. The saturated fatty acids in faecal fat followed more or less the solid fatty acid content of the blend. For blends with higher m.ps. chylomicron curve flattened and the maximum was shifted to the right indicating not only a slower absorption, but probably also a slower removal of fat from blood (Sahasrabudhe, 1954). The iodine value and saponification value of the faecal lipids of the rats fed different edible fats were almost the same and had similar fatty acid composition (Narayana Rao, 1955).

No marked differences were observed in fat storage, concentration of total cholesterol or total fatty acids in blood plasma, liver fat or vitamin A content of liver fat between rats fed a fat free diet, a diet containing 30 per cent refined groundnut oil, 30 per cent vanaspati or 30 per cent ghee. Calcium storage was less on the fat free diet (Bose and Subrahmanyan, 1950). The major component acids of the body fats of rats on fat free diet were oleic, palmitic, hexadecenoic acids and smaller amounts of stearic, myristic and linoleic and traces of arachidic and arachidonic acids. Feeding of groundnut oil substantially raised the linoleic acid content and slightly increased arachidonic acid. Higher amount of hexadecenoic acid was found in the fat free group. Iso-oleic acid was identified in the vanaspati group. The saturated acids, myristic, palmitic, stearic and arachidic were same in all. Lauric acid was found only in the group fed on cow ghee (Bose and Subrahmanyan, 1951). Fortification of hydrogenated groundnut oil with saturated fats and iso-oleic acids had little effect on the body weight increase over 12 weeks, the percentage of body fat or its content of saturated acids of the liver fat percentage. The digestibility of the fortified fat was slightly less than that of original fat, which may be due to the larger excretion of saturated acids, which was proportionally

greater than that of iso-oleic acid. During fasting the iso-oleic acid disappeared more readily than the saturated acids of the body. The digestibility of both the fortified and original fat increased with age of animal (Bose and Subrahmanyan, 1950*a*). Rats fed a poor rice diet with supplement of 30 per cent cow ghee had no deposits of lower fatty acids from butyric to capric, even though the ghee contained them to about 20 per cent. The major component acids of the liver fat were palmitic and oleic acids. (Bose and Subrahmanyan, 1952).

In fat deficiency, succinic, butyric and caproic dehydrogenases of rat liver were reduced and were further reduced when superimposed on vitamin B_6 deficiency. Glutamic dehydrogenase of the liver and the crude fatty acids of liver and their iodine value were also significantly reduced in the deficiency of fat and pyridoxine singly and in both. Total liver lipids decreased significantly in the combined deficiency of fat and pyridoxine (Tulpule and Patwardhan, 1952).

Rats on a high fat diet lost body weight, the loss being more in the absence of choline. Rats on a fat free diet maintained weight and had a much higher carcass fat. Choline affected the nature of the phospholipid fatty acids of the liver; the proportion of solid fatty acid was considerably higher in choline deficiency on both the high fat diet and the fat-free diet and the average unsaturation of the liquid acids was higher in the choline deficient group. There was no change due to choline deficiency in the nature of the fatty acids of carcass phospholipids or of glycerides from liver or carcass (Raman, 1952).

The presence of excess Ca in the diet reduced digestibility of the higher melting saturated acids, but did not influence the digestibility of the oil itself or its component liquid fatty acid fraction (Rao and De, 1951). On the other hand, both whole fat and saturated and unsaturated fractions given separately increased the percentage Ca utilsation by young growing rats (Rao and De, 1952). Of the steam-volatile fatty acids, butyric, caproic and caprylic acids permitted a low utilisation of Ca in rats (Narayana Rao, 1954*b*).

Rabbits fed cholesterol in the diet began to show after about three weeks, proliferation of the reticular connective tissue of the aortic intima and pulmonary artery, followed by deposition of cholesterol ester (Chakravarti *et al.*, 1955). There was not much difference in the serum cholesterol values in rabbits on the stock diet alone or with a daily extra fat supplement of 5 c.c. At autopsy, fatty infiltration of the lungs and heart was found in some animals in each group given extra fat; two of the rabbits given coconut oil had fatty livers; the aortic intima of two of the animals given groundnut oil, showed reticular proliferation with deposition of collagen (Chakravarti, *et al.*, 1956).

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PROTEINS AND AMINO ACIDS

PROTEINS

Nutritive value of proteins :

Studies on the nutritive value of different food proteins and the possibility of improving it have been engaging the attention of many workers for a number of years.

Dikshit and Patwardhan (1950) found that duck's egg white (DEW) has a low efficiency for promotion of growth in rats when compared with hen's egg white (HEW). Determination of essential amino acid composition of duck and hen egg whites by Patwardhan and Vijayaraghavan (1954) showed only minor differences between the two proteins ; these could not explain the low growth. Dikshit and Patwardhan (1954) found that the growth promoting property of both DEW and HEW varied according to season, both being highest in March or near about that time. DEW at 16 per cent protein level gave better growth than at 8 per cent level. Narasinga Rao and Patwardhan (1954) observed that the growth promoting capacity of DEW could be improved considerably and brought up to that of HEW by autoclaving the DEW at 15 lb. for 1 hour, which treatment, however, had no effect on HEW. The authors separated the monocoagulable growth inhibitor, from the coagulable proteins of DEW and found that the latter promoted growth better than whole DEW and to a degree comparable to that promoted by the coagulable proteins of HEW. The authors concluded that the inhibitor was an ovomucoid having the properties of a tryptic inhibitor. Vijayaraghavan and Narasinga Rao (1953 and 1954) found that *in vitro* liberation by trypsin of amino N as well as that of several essential amino acids from different proteins was adversely affected by DEW ovomucoid and hence the authors attributed the growth depressing action of duck ovomucoid to a possible block in the supply of essential amino acids for tissue protein formation. Narasinga Rao and Patwardhan (*loc. cit.*) purified the DEW and HEW ovomucoids and obtained them as electrophoretically homogenous samples. The DEW ovomucoid resisted destruction by heat and by tryptic digestion better than HEW ovomucoid. It also inhibited growth in rats when fed at 1 per cent level with different proteins at 10 per cent levels, whereas HEW ovomucoid had no such action. Even when fed with acid and enzyme hydrolysates of casein. DEW ovomucoid depressed growth in rats. Hence the authors suggested that the inhibitor had two-fold action, one as an antitryptic factor and the other as a growth inhibitor independent of the former and working through an yet unknown mechanism.

Rahaman and De (1950) have reported that varieties of soyabeans with a low antitryptic factor content had higher supplementary and biological values than those which were richer in the anti-tryptic factor. Viswanatha and De (1951) suggested that the low nutritive value of raw soyabean might be due to the slow liberation of methionine. Peptic digestion improved the availability of methionine in raw soyabean and it was postulated that this was due to the breakdown of protein to peptides the digestion of which by trypsin is unaffected by the inhibitor. They further observed that the liberation of methionine from germinated soyabean by pancreatin was faster in spite of the presence of inhibitor. Inhibitor-extracted and autoclaved soyabeans incubated with pancreatin had the same digestibility and availability of lysine and methionine which again suggested that during autoclaving only the inhibitor was destroyed and that there was no change in protein structure.

Appanna and Devadatta (1954) reported that prawn (*parapaeniopsis sculptilus*) pulp as the only source of protein and when fed at 7·5 per cent in the diet, produced loss of weight in mature rats and the skeletal development was poorest. With levels 15 per cent, 17·5 per cent and 20 per cent prawn pulp, no difference in skeletal development and in gain in weight was found and the composition (Ca : P) of the bone was not affected by the level of protein. Subrahmanyan *et al.* (1953) recovered casein from deteriorated skim milk powder and found its biological value by rat growth method to be 64·5± 1·57 compared to the casein from unspoiled milk which had the B.V. of 70·3± 1·83.

Balasubramanian *et al.* (1955) studied the nutritive value of the proteins of milk and some milk products like dahi, khoa, kheer, etc. and reported that the nutritive value of milk was improved in kheer. Subramanian *et al.* (1952) found that seed proteins of *Sesbania grandiflora* which contains nearly 70 per cent protein could not support by itself the growth of young rats, whereas mixed with casein in equal or half the amount, it gave improved protein efficiency ratios. Devadatta *et al.* (1951) reported that germination reduced the biological value of the proteins of cotton seeds, Bengal gram and lentil.

Narayana Rao *et al.* (1951) found that tapioca protein contained only 10·5 per cent N and no methionine. A basal diet containing 70 per cent tapioca as the sole source of protein could not support growth in young rats, whereas inclusion of Red gram, Bengal gram, soyabean or casein to raise the dietary protein to 10 per cent brought about continued growth, which was best with casein. Sweet potato, as the sole source of protein, was similar to tapioca, but with casein as supplement the growth was not as good as in the case of tapioca.

Lal and Rajagopalan (1953) reported that protein of low biological value in oil cakes could in some cases be improved by mixture with a cereal, yeast and groundnut. Rao *et al.* (1954) found that the protein efficiency ratio of bajra could be improved by the supplementation with proteins of groundnut cake and Bengal gram. Sur *et al.* (1954 and 1954*a*) reported that replacement of part of the ragi, milo or wheat in the diet by food yeast (*Torula utilis*) greatly stimulated growth of rats but there was only a slight effect in the case of rice.

Baliga and Rajagopalan (1954) reported that the biological value of cold defatted soyabean flour protein was considerably increased with supplementation by vitamin B_{12} or after a period of iodinated casein feeding. Both vitamin B_{12} and aureomycin were found by Baliga *et al.* (1954) to improve the biological value of defatted autoclaved soyabean meal. Vitamin B_{12} countered the effect of soyabean tryptic inhibitor when added to a casein diet whereas with soya bean, B.V. was improved by vitamin B_{12} whether the tryptic inhibitor was present or not.

Moorjani and Bhatia (1954) found that groundnuts stored for 18 months at 20°C. to 30°C. lost considerable amount of protein N, NaCl-soluble N and N in the NaCl extract precipitable by trichloracetic acid. The digestibility estimated by pepsin and trypsin digestion was reduced by 7-9 per cent and the protein had become partly denatured. Das *et al.* (1953) reported that the *in vitro* digestion of casein by trypsin or pepsin was slower after heating with sugars. The rates of digestion of the proteins of khoa prepared from skimmed milk powder or buffalo milk and cane sugar were only slightly lower than those of the original milk powder and milk.

Hydrolysis of proteins :

Gurnani and Sahasrabudhe (1953) and Gurnani *et al.* (1955) described a rapid method of hydrolysis of tissue proteins. The tissue was boiled with 85 per cent formic acid and 2 N HCl for 2 hours under reflux. Tryptophane was partly destroyed ; other amino acids were stable to this treatment. Formic acid treatment, however, had no effect on the rate of acid hydrolysis of gelatin and casein. Krishnamurthy (1952) reported that enzymic hydrolysates of casein had the same biological value for rats as casein because of their strepogenin content which was unaffected by hydrolysis.

Haemopoitic efficiency of proteins :

Mahalanobis and Roy (1952) found that in rats rendered anaemic by injections of phenylhydrazine, the rate of haemoglobin regeneration was slightly higher with protein supplied as boiled fish (*Ophicephalus*

punctatus Beng lata) than as skimmed milk powder. Vijayaraghavan (1955) found in rats rendered anaemic by phenylhydrazine, that at all levels, the animal proteins, such as casein, egg albumin, meat and liver proteins gave more rapid regeneration of R.B.C. and haemoglobin than vegetable proteins. The rate of regeneration increased with an increase in the level of dietary protein. The author found that the level at which N equilibrium was produced by a protein did not indicate its efficiency for haemopoiesis.

Supplementary value of protein to a poor diet :

Murthy *et al.* (1955) reported that 5 orphan girls aged 7 to 9 continuing to eat their usual poor rice diet with an average energy intake of 1010 calories and having a mean daily N intake of only 3·0 g. had adapted themselves to the low intake and were all in positive nitrogen balance on this intake. The effect of replacing rice by an equal quantity of a vermicelli type of food preparations containing jowar, wheat and tapioca flours in the proportion of 2 : 1 : 1 was studied in 21 children for 8 weeks by Subrahmanyan *et al.* (1954) who reported a highly significant increase in weight compared to the controls and also evidence of improved nutritional status in the experimental group. In yet another study, Reddy *et al.* (1954) found a composite protein food (Indian patent No. 47580, 1953) containing 22·4 g. protein per oz. besides minerals and B vitamins was highly nutritious when fed to 10 children for a period of 10 weeks. Sur *et al.* (1955) found that in undernourished children a daily supplement of 12 oz. groundnut milk curds to the basal poor rice diet significantly increased the retention of N and P. Subrahmanyan *et al.* (1955) observed in metabolic trials carried out on young adult subjects that the apparent digestibility of ragi protein was 50 per cent.

Effect of protein deficiency on enzymes :

Srinivasan and Patwardhan (1955) studied in detail the effect of protein deficiency on some liver constituents and enzymes in liver, pancreas and blood in albino rats. In adult rats liver fat showed a temporary increase after the second month of protein depletion, though there was no histological fatty infiltration. Glutathione fell in the first month and then remained low. Succinic acid and butyric acid dehydrogenases and transaminase were effected in the first month; D-amino acid oxidase and esterase in the second month. Alkaline phosphatase activity rose progressively until the third month in adult rats, whereas acid phosphatase increased only after 3 months. Oxygen consumption by liver cells fell in the first month and remained low. Realimentation with protein restored all the liver constituents to normal. Pancreatic lipase was

reduced in the third month and plasma lipase and esterase in the fourth month. The enzyme changes seen in adult rats in 3 months were already visible after 1 month in young rats on protein deficiency.

Metabolsim of proteins in humans :

Venkatachalam and Patwardhan (1953) found that the ascaris in intestines interfered with the digestion and absorption of protein. In 9 children infested with *Ascaris lumbricoides*, the faecal nitrogen, after deworming, showed a significant decrease which the authors observed was not due to the nitrogen content of the ascaris eggs or the anthelmintic. The authors suggested that this effect of the ascaris infestation should be considered important in the aetiology of nutritional oedema syndrome.

Ramamurti (1955) reported values for the nitrogen partition in the urine of Rajasthanis. Chitre *et al.* (1955) observed that in young Indians, both vegetarians and non-vegetarians, when the protein intake was raised, the urea clearance rose to values comparable with those reported for Americans. Of the N compounds in blood and urine, only urinary urea was increased on the high protein diet and there was no difference between animal and vegetable proteins in this respect. Studying the excretion of creatinine in urine, Karambelkar *et al.* (1952) found that in 4 out of 7 subjects on a vegetable protein diet there was a definite increase from 8 to 21 per cent when 41 to 49 per cent of the dietary protein was changed over to animal protein supplied in the form of eggs or milk in 2 subjects the increase was not statistically significant.

Protein requirements :

Detailed study has been carried out by Phansalkar and Patwardhan (1954, 1955 and 1956) on the metabolism of protein as affected by the sources of protein leading to the determination of minimum protein requirements for maintenance in young Indian men. The authors (1954) found that the 24-hour urinary N excretion in 15 Indian subjects on unrestricted diets was $7{\cdot}050 \pm 0{\cdot}906$, indicating a low level of protein metabolism. The composition of urine with respect to various nitrogenous constituents was found to be similar in these series and Folin's (1905) on comparable levels of total urinary N. Studies in three subjects on controlled diets revealed a high correlation between urea N and total N in urine similar to that in the Folins' series. The differences in urine N between animal protein and vegetable protein diets were accounted for by differences in urea N alone. Studying the excretion of urinary N in absorptive and early post-absorptive phases, Phansalkar and Patwardhan (1955) reported that from 11 to 35 per cent more urinary N was excreted

within 8 hours after the animal protein test meal than after the vegetable protein, although the intake of protein, fat and energy was constant in 7 healthy men. Urine volume was directly correlated with urine N; a large volume of urine was excreted after the animal protein than after the vegetable protein meals. Blood sugar rose and fell more quickly after the animal protein than after vegetable protein, although the peaks attained were identical. The same authors (1956) found that at marginal levels of protein intake, replacement of egg protein by a single cereal protein resulted in the body getting into negative N balance, whereas when replaced by mixed cereal and pulse proteins, urinary N was lower than on whole egg protein, confirming the observations of Patwardhan *et al.* (1949) and Karambelkar *et al.* (1950). The minimum levels of protein needed to maintain N equilibrium on vegetarian diets of pulses, cereals and vegetables was found to the 3·55 g. N m^2 body surface which value agreed with those reported by other authors in western subjects.

Gopalan (1956) studied in 14 poor class Indian infants the actual amounts of breast milk consumed in 24 hours, determined at weekly intervals for the first four months and fortnightly for the next 20 weeks. The average 24 hours milk output of the mothers ranged from 16 to 18·6 oz. and the average milk protein intake of the infants from 2 g./kg. body weight in the first fortnight to nearly 1 g./kg. in the sixth month. Comparing their growth with that of American children, it was found that though the actual weights were different, the growth rates were not widely divergent. Using the available data on body composition of infants, the author estimated the protein requirements (presumably human milk proteins) in infancy and suggested "not more than 2 g./kg. in the first few weeks and probably even less in the later stages".

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AMINO ACIDS

Giri *et al.* (1953) found that when samples of casein, egg albumin gelatin and fibrin in the presence of equal amount of glucose were moistened and autoclaved at 15 lb. pressure, loss of arginine was complete from casein, but less so from the other proteins after heat treatment for 1 hour ; about half of lysine and histidine was lost from all proteins after 2 hours and on extending the time for casein to 8 hours, slight destruction of tyrosine and cystine also occurred, but the other acids remained unaffected.

Wahi *et al.* (1954) reported that livers of albino rats on a normal stock diet contained cystine, aspartic acid, glutamic acid, serine-taurine glycine, alanine histidine, valine, leucine and tyrosine in the free form. These acids were also found in urine. Non-essential amino-acids except tyrosine were present in greater concentration.

Sadasivan (1950) observed that while choline, acting alone or in combination with cystine, showed marked reduction in liver fat in rats on a high fat low protein diet, methionine at the level of 100 mg. per cent did not lower the same. Cystine alone showed markedly increased deposition of fat in liver. All the three depressed the excretion of urinary N, P and S. Chowdhury and Chakrabarty (1952) reported that 13 persons suffering from liver disease excreted higher amount of methionine ranging from 350 to 500 mg. per day as compared to the 17 normal Indians in whom urinary excretion of methionine was from 150 to 285 mg. per day.

Sundaram and Sarma, (1953) and Sundaram *et al.* (1953) reported that the yellow pigment excreted by pyridoxine deficient rice moth larvae (*Corcyra Cephalonica St*) receiving DL-trytophane consisted of kynurenine and 3-hydroxkynurenine both of which were absent when the larvae received pyridoxine. Feeding of DL-kynurenine also gave yellow faeces and it was concluded that unlike mammals the larvae did not convert kynurenine to zanthuranic acid. Shanmugasundaram and Sarma (1955) found no normal tryptophane metabolite in biotin-deficient rats and suggested that biotin was necessary for tryptophane metabolism, at the stage when tryptophane is degraded to formylkynurenine.

Chandran and Damodaran (1951) reported that the rates of regeneration of r.b.c. and haemoglobin formation in anaemic rats on a basal iso-leucine-free diet containing vitamins and 18 per cent horse globin as the sole source of protein was significantly higher when 20 to 100 mg. L-isoleucine by mouth or 20 mg. subcutaneously were given daily than on the basal diet alone or when getting a daily supplement of only 10 mg. isoleucine. Oral administration of isoleucine was more effective

for haemoglobin formation than that given parenterally although the recovery time was similar. Acute anaemia could be produced in normal rats after 40 days on a isoleucine-deficient diet, which could be cured by daily oral feeding of 20 mg. isoleucine for 15 days.

Sivaramakrishnan and Sarma (1955) observed that sulphanilamide added to solution of radioactive histidine in which green gram seeds were allowed to germinate for 72 hours, inhibited the metabolism of histidine. From simultaneous determinations of activity in glutamic acid isolated from the seeds the authors concluded that conversion to glutamic acid of histidine was not the main pathway of histidine metabolism.

Dasgupta and Basu (1954) have studied the metabolism of amino-acids in heart and lung tissues of rats, and have found that in both heart and lung l-isoleucine and l-valine in heart tissues only d-histidine and in lung tissues only d and dl-isoleucine and d-valine suffered both oxidation and deamination. Cysteine was oxidised without ammonia production by the heart tissue.

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MINERALS

Calcium and phosphorus.—Sur and Subrahmanyan (1952 and 1955) found that lucerne leaf contained calcium to the extent of 2 per cent on dry basis, which was nearly as well utilised by the albino rat as that in whole milk, unlike that in amaranth. Iengar and Rau (1952) have pointed out that value of a vegetable as a source of dietary calcium would depend not only on its calcium content, but on its oxalic acid content as well. More calcium would be available if the ratio, calcium : oxalic acid was greater than unity. Sathe *et al.* (1952) found that polishing rice to 7 per cent gave optimum values for Ca and P utilisation by growing rats as compared with milled rice. Such rice contained 1·8 μg. thiamine per gram, as recommended by the FAO Nutrition Committee for South East Asia.

Iron.—Agarwal and Misra (1955) found that in anaemia cases serum Fe and saturated Fe were low. Subrahmanyan *et al.* (1950) reported that 90 per cent of the ionisable Fe in autoclaved soyabean was utilised for haemoglobin formation. The soya milk Fe was found to be utilised to the same extent as in $FeCl_3$. Sathe and Krishnamurthy (1953) found that the organic acids, such as tartaric acid, ascorbic acid or citric acid, improved the availability of Fe in soyabeans for haemoglobin regeneration in rats rendered anaemic on a milk diet. They also found that the utilization of the Fe from ferric citrate for haemoglobin regeneration was most rapid and the storage of Fe higher in the group receiving 15 per cent polished rice than in that receiving unpolished rice, and concluded that phytic acid P interfered with the absorption of Fe. De (1950) conducted metabolic studies on six normal adults for a study of iron metabolism with typical Indian dietaries. Rice diets provided 36·3 mg. Fe and wheat diets 44·0 mg. and the retentions were 8·06 and 7·82 mg. respectively. Variations in Fe intake did not affect urinary output which on an average was 0·60—0·62 mg. per day on all diets. Experiments on low-Fe diets indicated that on 10 mg. Fe daily the subject was in positive balance. The minimum requirement of Fe has been stipulated by the author at 9 mg./adult/day. He observed an increased retention of Fe when the intake increased from 25 to 37 mg./day and thereafter the retention remained constant at about 9 mg./day.

Zinc.—In the course of his studies on the biochemistry of zinc, Sadasivan (1951) found that ZnO fed at a level of 0·5 per cent reduced the body gain in the rats slightly, whereas at a 1 per cent level both the food intake and weight gain were reduced. The total femur ash weight and the percentage ash in the dry femur were reduced by the ZnO intake. Zinc oxide in a high-fat low-protein diet for young rats, acted as a

lipotropic factor. The same author (1951 and 1952) found that the inclusion of ZnO in the diet increased both the urinary and faecal N with consequent decrease in N-balance. The urinary excretions of both S and P decreased, but these decreases were counterbalanced by the increased faecal excretions. Uric acid and creatinine in urine were also increased. The phosphatase activity in the intestine was reduced, whereas the activity in liver and kidney increased.

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VITAMIN A AND CAROTENE

De (1955) has described a fluorometric method for vitamin A assay based on the observation that vitamin A alcohol in light petroleum exposed to light at 365 mμ gives a green fluorescence. A linear relationship between fluorescence and concentration was obtained for the range 1 to 18 I.U. of vitamin A. Good agreement was found between the values obtained by the fluorometric, spectrophotometric and colorimetric methods. Ultraviolet irradiation of carotene produced a fluorescent material but the product was not identical with vitamin A. Balasundaram *et al.* (1955) estimated spectroscopically and colorimetrically the content of vitamin A_1 and A_2 in the liver oils of three species of Indian fresh water fish and reported that the vitamin A_2 content exceeded by nine times the amount of vitamin A_1 found in these fishes. Most of the vitamin A_2 in the oil was found to be in the ester form with a small quantity present as alcohol in each of the oils. Pradhan and Magar (1956) reported that in the liver oils of 5 species of sharks investigated, neovitamin A formed 19·86 to 32·51 per cent of the total vitamin A ; the ratio of vitamin A_1 : A_2 varied between 12 : 1 and 25 : 1, the vitamin A_2 alone constituting 4 to 8 per cent of the total vitamin.

Rajagopalan and Datta (1952) studied the reaction of vitamin A aldehyde with plasma protein. Vitamin A aldehyde prepared from synthetic vitamin A acetate was added in alcoholic solution to a solution of plasma albumin prepared from bovine plasma and dialysed upon which the solution gave an absorption spectrum having two maxima, one at 280 mμ for the albumin and the other at 378 mμ due to the aldehyde-protein compound. In the condensation product, 1 g. of protein was associated with 30 μg. of vitamin A aldehyde, which is roughly equivalent to five times the concentration of vitamin A alcohol present in normal blood.

Patel *et al.* (1955) found that both vitamin A ester and alcohol were less stable in aqueous media than in oils with or without antioxidant. In fatty media, ester was more stable to oxidation than alcohol. Bhatia (1952) reported that every cooking process involved a loss of vitamin A varying from 20 to 100 per cent. Total loss occurred on frying for more than 15 minutes. Shroff *et al.* (1954) reported that after heating for 1 hour at 125°C. and 150°C. the vitamin A losses in ghee were 11 and 82 per cent respectively and at higher temperatures the losses reached 85 to 100 per cent in 10 minutes. De and Kshirsagar (1954) found the loss of vitamin A during various processes of cooking to depend upon the duration of heating, maximum temperature reached, size of the vessel used, the quantity cooked and the addition of

extraneous water etc. The authors considered that despite the large loss of vitamin A when fortified vanaspati is used for cooking, the amount remaining makes a substantial contribution to the diet. Studying the nutritive value of vanaspati, Chitre and Khale (1956) reported that the vanaspati fortified with synthetic vitamin A palmitate and stored in 1 lb. tins open to atmosphere at room temperature lost 25 per cent of the vitamin in 6 weeks whereas preserved in sealed tins, it required 6 months for the same proportion of loss to occur. Chitre and Khale (1956) also found that the vitamin A palmitate in the fortified vanaspati was equally well available biologically as that in cod-liver oil. Kalyanakrishnan *et al.* (1951) reported that supplementation to cows and buffaloes on a low carotene diet, vitamin A concentrate of total value of 14,00,000 I.U. per head over a period of 10 days, increased the vitamin A content of the butter-fat substantially. Narayanan *et al.* (1954) found that in milk fortified with water-miscible vitamin A, the vitamin remained in the aqueous phase after skimming, whereas in the milk of lactating cows fed the aqueous preparation of vitamin A, all the vitamin was found only in the fat fraction and not in the skim milk.

Esh and Bhattacharya (1951 and 1953) found that when rats were given massive doses of vitamin A alcohol, maximum liver storage of the vitamin occurred when the supplement was fed in aqueous medium ; next in efficiency were the media ethyl oleate and arachis oil in that order. For vitamin A acetate, however, maximum storage occurred when given in arachis oil, and for vitamin A palmitate, aqueous medium was the best. The highest storage from all the media was found to be with vitamin A palmitate compared to vitamin A alcohol or acetate fed. With α-tocopherol, lecithin or propyl gallate, significant increase in liver storage was obtained from arachis oil and ethyl oleate as solvents, but not in aqueous media. Krishnamurthy and Ganguli (1956) found that in rats given a daily intraperitoneal injection of a 20 per cent suspension of Indian ink in normal saline previously for a few days, and a single high oral dose of vitamin A on the last day of the injection, there was low concentration of vitamin A ester in liver and a high level of the vitamin in blood than in rats given only saline, showing thereby that blocking the reticulo-endothelial system, interfered with the storage of the ester in the liver while not affecting the alcohol which fact suggested that there are two different mechanisms for storing the two forms of the vitamin.

Chattopadhyay and Banerjee (1951) reported that in seeds of pulses, wheat, maize and paddy rice, there was a steady increase in the carotene content in the first few days after germination. Karmarkar *et al.* (1952) studied the stability of carotene concentrate prepared from

leaves of the water hyacinth and dissolved in arachis oil and in mustard oil. The loss of carotene was greater at higher temperature and the percentage loss was greatest with lowest initial concentration. Of the antioxidants, wheat germ oil, dl-methionine and α-tocopherol, the last named had the maximum effect in preventing the loss of carotene. Working with rats, De and Sundararajan (1951) found that 24 hours after oral administration of carotene, the small intestine was almost devoid of carotene and the intestinal bumen contained no vitamin A in most of the experiments, whereas the intestinal wall contained appreciable amounts. They concluded that the wall of the small intestine was the site of conversion of carotene to vitamin A. However, in experiments *in vitro* in which excised intestinal loops were incubated with different amounts of carotene under various conditions no evidence was obtained of the conversion of carotene to vitamin A.

Dikshit and Ranganathan (1955) in their studies on carotene metabolism in mammals, found that goats on a diet of green grass, cabbage leaves and Bengal gram excreted substantial amounts of carotene in faeces, but had no trace of carotene in blood or milk. After massive doses of 5,000 to 14,000 μg of carotene by oral, subcutaneous or intravenous route, the vitamin A content of milk was raised substantially, although no trace of carotene was found in milk or blood. Intravenous injection of a massive dose of water-miscible preparation of vitamin A increased the content of vitamin A in both milk and blood significantly. *In vitro* studies in which carotene was incubated with goat's blood suggested that carotene was destroyed by oxidation and not by enzymic action. About 60 per cent of intravenously administered carotene could be accounted for in the lungs within 10 minutes and none in the blood. Colostrum contained no carotene, but was rich in vitamin A. The authors (1955) found in experiments with rats that after an intramuscular injection of a massive dose of carotene only a trace was found in lungs after 24 hours. When smaller amounts were injected into the heart, considerable amounts of carotene were found in lungs. After injection of carotene direct into the spleen so as to avoid its having to pass through the lungs before reaching the liver, distinctly discernible amounts of carotene could be found in lungs contrary to expectations. They also reported that carotene was physiologically available when given to vitamin A deficient rats by the intramuscular, intracardiac or intrasplenic routes.

Datta and Rajagopal (1952) found that during certain pathological conditions, vitamin A was excreted along with urine. Of 21 urine samples from patients with pulmonary tuberculosis, only one contained vitamin A, 18 I.U./100 c.c. and out of 28 cases of lobar pneumonia 16

contained detectable amounts of the vitamin. No parallelism was observed between the amounts of vitamin A excreted and the amount of protein or purines excreted or the W.B.C. count. When the urine of the above patients or freshly voided urine of normals was shaken with the non-saponifiable fraction of halibut liver oil, most specimens of urine took up measurable quantities of vitamin A. The effect was not observed, however, when the urine became denatured on standing for 24 hours or when the pH was altered ; boiling for 10 minutes under reflux completely prevented solution of vitamin A. Addition of xanthine and hypoxanthine slightly increased the solubility of vitamin A, but adenine and guanine had no effect.

Agarwal and Adhaulia (1954) reported that healing of superficial or deep wound made on the cornea of rabbits was quicker in animals given daily an intramuscular injection of 10,000 I.U. vitamin A than in untreated controls. In human subjects with corneal ulcers, administration of vitamin A by intramuscular route besides routine treatment hastened the healing time in days. The density of the scar was less in the patients who received vitamin A.

Nerurkar and Sahasrabudhe (1956) found that hypervitaminosis A resulted in negative balances of Ca, P and N which were corrected during the subsequent period of two weeks of normal vitamin A intake. Excess of vitamin A reduced food intake and there was loss of weight with skeletal fractures and haemorrhages in the young rats. There was no change in the concentration of Ca and inorganic P in blood. Changes in the composition of bones were small, so that the loss of Ca and P was probably by thinning of the bones. Liver storage of vitamin A showed absorption to be poor when given in amounts far exceeding the normal requirement. Rahalkar *et al.* (1956) found that in hypervitaminosis A alkaline phosphatase in marrow, plasma, liver, kidneys and intestines showed substantial increase.

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VITAMIN B_1

Balakrishnan and co-workers have studied in great detail the intestinal synthesis of thiamine as affected by various dietary factors. Diets low in protein, phosphorus and vitamin B_1 seemed to promote the intestinal synthesis of the latter in albino rats (Balakrishnan and De, 1951 and Balakrishnan and Rajagopalan, 1954). The quality of protein, however, seemed to have no effect on the intestinal synthesis of the Vitamin (Balakrishnan and De, 1951). Rats receiving butterfat in the diet grew better, excreted more vitamin B_1, had a higher liver vitamin B_1 content and a higher faecal bacterial count than the rats receiving coconut or groundnut oil (Balakrishnan and Rajagopalan, 1953). Supplementation of penicillin to young rats resulted in a better growth and higher liver stores of vitamin B_1. The coliform counts in faecal and caecal flora did not change suggesting that the antibiotic did not promote higher intestinal biosynthesis of vitamin B_1 but only suppressed the growth of micro-organisms utilizing vitamin B_1, thus making available more of the vitamin to the animal (Balakrishnan and Rajagopalan 1954). Feeding thiamine through curds influenced the thiamine synthesis in the intestine to a significantly higher extent than through milk or as synthetic vitamin (Balakrishnan and Rajagopalan, 1954 and 1954*a*). Sulphaguanidine included in a vitamin B_1—deficient diet fed to rats, reduced the urinary and faecal excretion of vitamin B_1. This reduction in the excretion of the vitamin due to sulphaguanidine, was much greater in rats receiving daily 10 μg. synthetic vitamin alone, than with 5 μg. synthetic vitamin plus 5 μg. vitamin B_1 from curds (Balakrishnan and Rajagopalan, 1953). Daily supplementation of 0·5 lb. curd providing extra protein energy and about 90 μg. vitamin B_1 and other B-vitamins to children on a poor rice diet, greatly increased their weight gain in two months and an increase in faecal and urinary excretion of vitamin B_1 attributed to greater synthesis in the gut, was also obtained. On the other hand; no such change was observed in children without the supplement or in those having daily a supplement of 90 μg. synthetic vitamin B_1, in 1 gm. of sugar (Balakrishnan and Rajagopalan, 1955).

Balakrishnan and Rajagopalan (1955*a*) also reported, that in rats, the highest weight gain, urinary excretion of vitamin B_1, liver content of vitamin B_1 and total counts of faecal flora, were found in those fed the whole wheat vegetarian diet of central India and Punjab. The results for ragi and rice diet of Mysore were almost as good. Gain in weight and vitamin B_1 synthesis were poor with rice and fish diet of Bengal and the vegetarian rice diet of Madras.

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Nath and Chakrabarti (1953) observed that in rabbits on germinated gram (*Cicer arietinum*) diet, intraperitoneal injection of sodium aceto-acetate or β—hydroxybutyrate increased the excretion of pyruvate and decreased that of vitamin B_1 in urine. In blood, a large increase in pyruvate and a decrease in vitamin B_1 were found after 90 daily doses of the supplement. Administration of vitamin B_1 restored the values for pyruvate in the blood and urine to normal. Incubation at pH 7·4 of Na aceto-acetate along with Vitamin B_1 destroyed the vitamin to a considerable extent whereas Sodium β—hydroxybutyrate had no such destruction effect *in vitro*, though it was as harmful as aceto-acetate *in vivo*. Saxena *et al.* (1954) reported that in cultures of freshly isolated strains of *bacterium coli*, addition of vitamin B_{12}, while not affecting the growth of the cultures, inhibited synthesis of vitamin B_1.

Sivaramakrishnan and Sarma (1955) found that when green gram seeds were germinated in a medium containing neopyrithiamine, the content of asparagine was less, glutamine slightly greater and free ammonia much greater, than in seeds without neopyrithiamine. The disturbance of the N-metabolism was partly reversed if vitamin B_1 and neopyrithiamine were present together and almost completely reversed if cocarboxylase was present. Injection of glutamate in rats given neopyrithiamine, precipitated acute signs of vitamin B_1 deficiency. Reddi (1951) reported that mustard seeds (*Brassica juncea*) contained a thermostable water soluble anti-thiamine factor which destroyed thiamine instantaneously between pH 2·7 and 7·5. The action was considered to be nonenzymic.

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RIBOFLAVIN

Sreenivasamurthy and Iya (1952 and 1953) reported that *Lactobacillus plantarum*—90 was superior to *Lactobacillus casei* as a test organism for the assay of riboflavin, since it gave a linear response to the vitamin over a wider range : it also produced a higher total acidity for titration as a measurement of growth and was not affected by the presence of protein and fat in milk.

De and Roy (1951) found that faecal and urinary excretion of riboflavin in adult rats on a riboflavin free regimen, were least in those receiving the dietary carbohydrate as sucrose and were higher in those receiving it as starch and lactose in that order. Sulphaguanidine depressed the faecal excretion of riboflavin, most in those fed lactose and least in those fed sucrose. The authors suggested that lactose promoted intestinal synthesis of the vitamin more efficiently than starch or sucrose probably by providing a more favourable medium for the intestinal flora. The riboflavin synthesized in the intestine was eliminated mainly with faeces, unabsorbed. The decreased excretion of urinary riboflavin with the high protein diet observed by De and Roy (1951) was attributed partly to decreased intestinal synthesis and partly to higher retention of the endogenous supplies from liver and kidney.

Nath and Chakrabarti (1953) suggested that the low value of riboflavin and nicotinic acid in blood and urine of rabbits given daily intravenous injection of Na aceto-acetate or Na-β—hydroxybutyrate, might have been due to destruction of the vitamin by accumulated ketone bodies or to excess demand in the metabolism of ketone bodies for the co-enzyme containing the two vitamins as prosthetic groups.

Giri and Krishnaswamy (1954) obtained from a bottom strain of brewor's yeast, a mutant top strain of *Saccharomyces cerevisiae* which was capable of excreting large amounts of riboflavin into the culture medium. The authors found that presence of adenine, guanine, xanthine, hypoxanthine, thymine or uracil increased the production of riboflavin by the yeast, but did not increase growth ; uric acid inhibited riboflavin synthesis. Tryptophan, phenylalanine or serine inhibited both the growth and the vitamin synthesis whereas methionine, glycine and arginine increased both.

De *et al.* (1955) found that in normal male Indians, of a daily riboflavin intake of 0·83 to 1·35 mg. in the vegetarians, 8·5 to 19·8 per cent was excreted in the urine and in non-vegetarians who had intakes ranging from 0·94 to 1·14 mg. riboflavin daily, 18·2 to 26·8 per cent was excreted. After a two-week period of saturation with a

daily dose of 10 mg. riboflavin the excretion in urine of a 5 mg. test dose of the vitamin was found to be about 50 per cent. This return of the test dose in the urine was found to remain constant at about the same level after the subsequent experimental periods wherein the subjects daily received 2 mg., 1 mg. dose and no dose of vitamin while on the basal experimental diets. Hence the authors concluded that typical Indian dietaries based on cereals with and without animal protein supplying 0·83 mg. to 1·35 mg. riboflavin daily were adequate to meet the minimum requirement for the vitamin.

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NICOTINIC ACID

Studies on the biosynthesis of nicotinic acid have been engaging the attention of many workers for some years. Banerjee *et al.* (1951) reported that on germination of pulses at 30°C, nicotinic acid, nicotinuric acid and N^1—methyl-nicotinamide (N^1-MN) increased greatly with a corresponding decrease in trigonellin which was in relatively large amounts in the ungerminated seeds. Nicotinic acid and nicotinuric acid increased on germination in cereals, but less than in pulses. The increased formation of nicotinic acid during germination of pulses was attributed to a possible conversion of trigonellin in N^1-methyl-nicotinamide with a subsequent demethylation. With cereals, which contain no N^1-MN or trigonellin either before or after germination, the synthesis of nicotinic acid during germination was suggested to be of a different kind. Ghosh *et al.* (1951) found that alkaline extraction of the muscle of the tortoise or of fish gave much higher values for nicotinic acid than aqueous or acid extraction which was not the case however, with beef and goat muscle. Hence they concluded that the bound form of nicotinic acid present in fish and tortoise muscle was different from that in cereals and appeared to be in combination with proteins and carbohydrates.

De and Datta (1951) reported that in adult rats faecal excretion of nicotinic acid on a protein and nicotinic acid-free diet, was the same as when the diet was supplemented with sulphaguanidine, tryptophan or both, whereas urinary excretion was less with sulphaguanidine and higher with tryptophan, which indicated that tryptophan was converted to nicotinic acid mainly in the intestine by the flora and that the vitamin synthesised was absorbed and used. Argenine, proline, asparagine or methionine in the diet did not increase the synthesis of nicotinic acid, nor prolonged deprivation of protein had any adverse effect on its synthesis. De and Guha (1951) found that adult rats on a protein and nicotinic acid deficient diet excreted in the urine a significantly less amount of nicotinic acid when the carbohydrate in the diet was sucrose than when it was, starch, dextrose dextrin or agar. Nicotinic acid excretion was higher in diets which included KNO_3. The same authors (1951) further reported that L-tryptophan stimulated synthesis of nicotinic acid in liver and kidney slices *in vitro*, whereas proline had no effect. Both liver and kidney tissues converted ammonium ion from added ammonium sulphate to nicotinic acid, but were unable to use nitrate ion from KNO_3. Vitamin B_6 or folic acid with tryptophan greatly stimulated the synthesis of nicotinic acid, but vitamin B_1 did not. They felt that vitamin B_6 transformed into pyridoxal, acted as the prosthetic group in a transaminating or deamination

enzyme system. Shanmuga Sundaram and Sarma (1954) observed that rice moth larvae fed on pyridoxine deficient diets grew less and had less niacin in their bodies. In experiments using DL-kyneurenine and 3-hydroxyanthranilic acid, the authors proved further that pyridoxine is required for conversion of tryptophan to niacin at the stage 3-hydroxykyneurenine—3-hydroxyanthranilic acid in rice moth larvae. De *et al.* (1951) reported that the return in the urine of a test dose of nicotinic acid was not affected in any way be administration along with the vitamin of sulphaguanidine, sulphapyridine sulphathiazole or phthalylsulphathiazole. But the drugs given over a longer period decreased the excretion of nicotinic acid and riboflavin. This suggested that the drugs inhibited microbial synthesis and did not interfere with absorption or utilisation. Sulphadimethylpyridine or sulphadiazine increased the excretion of nicotinic acid and riboflavin possibly by inhibiting growth of intestinal organisms utilising nicotinic acid and riboflavin. Paludrine, quinine sulphate and calcium salt or penicillin decreased the excretion of nicotinic acid.

Shanmuga Sundaram and Sarma (1953) showed that rat liver synthesised *in vitro* nicotinic acid from D-tryptophan and the percentage of utilisation was higher at lower levels of tryptophan, whereas cattle and rabbit livers did not synthesise it. At higher levels, the normal synthesis of niacin was inhibited by the D-isomer. The utilization of the isomer was ascribed to the presence of D-amino acid oxidase in rat liver and to its absence in the liver of cattle and rabbit or in germinating green gram. Subrahmanyan (1954) reported that the biological value of the dietary protein low in tryptophan, was enhanced by added tryptophan and the effect was further increased by giving nicotinic acid with a small supplement of tryptophan. With adequate addition of tryptophan, nicotinic acid had no further effect. Subrahmanyan (1954) also observed that liver slices of rats fed on a vitamin B-complex deficient regimen, when incubated in phosphate buffer with tryptophan, could not convert the amino acid into nicotinic acid. Only in the presence of pyridoxine any considerable conversion of tryptophan to nicotinic acid occurred. Shanmuga Sundaram and Sarma, (1954) reported that during germination of green gram, the γ-3: 4-ureylene cyclohexyl butyric acid, the anti-biotin, inhibited biosynthesis of nicotinic acid, which effect could be overcome by excess of the biotin : aminopterin also inhibited the biosynthesis which was reversed by excess of folic acid, whereas ω-methyl pantothenic acid had no influence on niacin synthesis. Hence it was concluded that biotin and folic acid were involved in the synthesis of nicotinic acid from tryptophan, biotin probably influencing the stage of conversion of tryptophan to kyneurenine. Ghosh *et al.* (1954) observed that in young rats on a niacin-deficient DL-tryptophan supplemented

regimen, daily intraperitoneal injection of carbon tetrachloride for one week resulted in a considerable reduction in the urinary excretion of quinolinic acid, N-Methyl-nicotinamide and nicotinic acid.

Sundaram *et al.* (1954) demonstrated that the excreta of rice moth larvae on diets containing nicotinic acid and nicotinamide contained only nicotinic acid. The larvae lacked capacity to amidate or methylate nicotinic acid but on the contrary it deamidated the amide. From a study of the urinary excretion of nicotinic acid derivatives before and after an intraperitoneal administration of 50 mg. nicotinic acid in rabbits on a diet free from N^1—MN and 1-methyl-3 carboxylamide-6-pyridone Chattopadhyay *et al.* (1953) concluded that while 6-pyridone of N^1—MN is the chief metabolite of nicotinic acid in rabbits, nicotinuric acid also is normally excreted : and that trigonellin is not a metabolite of nicotinic acid in the rabbit. Banerjee and Basak (1955) found that in female rhesus monkeys receiving an adequate diet, administration of sulphaguanidine slightly decreased the urinary excretion of N^1—MN, but no change in nicotinic acid or amide, nicotinuric acid, 6-pyridone, trigonellin and quinolinic acid. Administration of nicotinic acid or tryptophan increased the excretion of N^1—MN, 6-pyridone and quinolinic acid, whereas trigonellin was not affected, apparently indicating that trigonellin was not an end product of nicotinic acid metabolism in monkeys.

Rindani and Narayan Rao (1950) reported that in diabetic patients, the excretion of nicotinic acid was within normal limits. Sen and Sen (1954) found that intramuscular injection of 100 mg. nicotinic acid in healthy male rabbits caused a rise in blood sugar and calcium accompanied as expected by a fall in inorganic phosphorus. The rise in Calcium could be explained as a compensating or associated phenomenon. Lower doses of niacin failed to produce any significant changes in blood sugar or calcium.

Agarwal and Dutt (1954) reported that in rabbits kept on a diet adequate in nicotinic acid, supplementation of 50 mg. nicotinic acid daily, hastened the complete healing of superficial or severe wounds made in the cornea. In patients with corneal ulcers given routine treatment, daily intramuscular injection of 50 mg. nicotinic acid, or 25 mg. subconjunctivally on alternate days, hastened the healing time, the latter treatment having a better effect. Scar formation was reduced with nicotinic acid treatment.

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VITAMIN B_{12} AND FOLIC ACID

The vitamin B_{12} activity of human milk and milk from animals have been estimated microbiologically by Sreenivasamurthy *et al.* (1950, 1953) using *Lactobacillus lactis* Dorner, as the test organism. Ewe's milk had the highest activity ranging from 4 to 20 mμg./cc. and human milk had the lowest total B_{12} activity, but the greater portion in the latter was cyanocobalamin. Higher values for total activity with much hydroxocobalamin included, was obtained for cow and buffalo milk. The values of B-complex vitamins including folic acid in buffalo milk as assayed microbiologically have been reported by Boman (1953). De and Mukerji (1951) evaluated the anti-anaemic potency of many samples of liver extracts of Indian and foreign manufacturers using the microbiological assay of vitamin B_{12} by *L. lactis*. Shenoy and Ramasarma (1954) reported that the addition of sodium metabisulphite following the papain digestion of homogenised liver tissue, had a protecting effect on the vitamin B_{12} activity during the subsequent heating. The same authors (1955) also reported that while aqueous solutions of crystalline vitamin B_{12} and B_{12b} could not be stabilised by the addition of $FeCl_3$, the vitamin B_{12} in liver extracts as well as the crystalline vitamin added to it, could be protected from destruction by heat at pH 10 by the addition of iron, Sreenivasamurthy *et al.* (1955) estimated microbiologically with *L. Leichmannii* 4797 hydroxocobalamin and cyanocobalamin and the bound and free forms of the vitamin in goat and sheep muscle, goat liver, fish and eggs. Goat liver was a rich source of vitamin B_{12} with 526 mμg./g. tissue nearly all of which was in the free form. Sheep muscle also was a good source with 300 mμg./g, tissue, but only 94 mμg. in the free form. Hence egg had 104 mμg./g. with 29 mμg. in the free form and fish had values ranging from 50 to 210 mμg./g. of which from 38 to 81 mμg. in the unbound form.

Dixit *et al.* (1956) have described a modified method of the microbiological assay of serum vitamin B_{12} using *Euglena gracilis* as the test organism. They (1956) determined the serum vitamin B_{12} concentrations in normal Indians and found an average value of 592 $\mu\mu$g./ml. with a range of 72-2160 in military personnel as against an average of 259 $\mu\mu$g./ml. and a range of 92-800, in the general public consisting of doctors, students, technicians, etc. It was suggested that the higher values in the men from the services could be due to the higher animal protein content of their diet.

Bhagvat and Sohonie (1955) reported that on a corn soyabean meal, rice moth larvae grew better if vitamin B_{12} was added. Sahasrabudhe and Rao (1951) observed that during repletion of fasted rats

administration of 3 μg. vitamin B_{12} intraperitoneally, increased the rate of liver protein regeneration and also of regeneration of RNA and DNA. They suggested that the effect of vitamin B_{12} on the DNA content of liver was particularly interesting since DNA was not affected by starvation and others have found that wide variations in protein intake did not change the amount. Ramalingaswami and Sriramachari (1953) found that vitamin B_{12} had no favourable effect on the toxic condition produced by oral feeding of the anti-malarial drug pyrimethamine (Daraprim) in the monkey and concluded that the effect of liver extract in alleviating the toxic effect of the drug could not have been due to its vitamin B_{12} content.

Fatterpaker *et al.* (1955) reported that while vitamin B_{12} had no effect on the glutathione content of liver, adrenal glands or blood of rats on a normal basal diet, it prevented the lowering of the values in the tissues and elevation in blood and the impairment of acetylating mechanisms in rats rendered hyperthyroid by feeding a diet containing iodinated casein.

Sreenivasamurthy *et al.* (1956) observed that in the liver of young rats born of vitamin B_{12} deficient mothers and maintained for 12 weeks on a B_{12}-free basal diet, the activity of lactic, glutamic, succinic, butyric and choline dehydrogenases, was reduced compared to B_{12}-fed controls; liver weights and liver N were not affected though the growth was higher in B_{12}-fed controls. Addition of vitamin B_{12} to liver homogenates of B_{12}-deficient rats *in vitro* did not raise the dehydrogenase activities to equal those of normal livers.

Fatterpaker and Sreenivasan (1951) showed that folic acid stimulated the process of xanthine oxidization *in vitro* in the presence of catalase upto concentrations of 60 to 300 mμg. folic acid per cent ; very high concentration of the order of 1·5 g. per cent inhibited xanthine oxidase. Fatterpaker, Marfatia and Sreenivasan (1951, 1955) found that synthesis of creatine *in vivo* and *in vitro* in adult Swiss mice was decreased in deficiency of folic acid or of vitamin B_{12}. The same authors (1952, 1955) later reported that 1 mg. methanol intraperitoneally daily was almost as effective as 10 μg. folic acid orally is repairing changes in creatine metabolism brought about by folic acid deficiency. Sodium formate in combination with folic acid, enhanced the effect of the latter on creatine metabolism to a slight extent, though formate alone had little effect. Folic acid and methanol together increased the synthesis of creatine *in vitro* in liver slices and increased creatine in muscle and urine. Subsequently the authors (1955) reported that in folic acid deficient or normal mice, supplementation of methanol and serine, did not cause

any change in the liver levels of choline, methionine and phosphocreatine. However, replacement of serine by glycine, resulted in higher choline-synthesis in liver when folic acid was given. The effect of vitamin B_{12} on methyl synthesis from precursors, methanol and serine was seen in both folic acid—deficient and folic acid-fed groups, while the effect of folic acid was seen only in the vitamin B_{12} fed group and not in vitamin B_{12}-deficient group. Hence it was concluded that while folic acid might be necessary for biosynthesis of ethanolamine moiety of choline, methyl synthesis from precursors was influenced more directly by vitamin B_{12}.

Fatterpaker *et al.* (1952, 1955) found that the urinary excretion of N-methyl-nicotinamide was significantly higher in adult mice receiving daily 10 mg. folic acid orally, or 1 mg. methanol or formate intraperitoneally or both, than in those receiving the basal folic acid-free diet. Nicotinamide methylkinase activity also was high in the supplemented groups. The conclusion that dietary folic acid influenced methylation of nicotinamide *in vitro* and *in vivo* was arrived at. The authors (1955) observed that in folic acid deficiency, there was an increase in the weight of liver, kidney and adrenal glands, the increases being greater when excess of nicotinamide was given. The effects on liver of nicotinamide toxicity combined with folic acid deficiency included increased neutral fat, decreased amount of choline and methionine, decreases in choline oxidase and to a slight extent in xanthine oxidase activity and on adrenals increased adrenaline content, all of which could be overcome by doses of 100 mg. folic acid daily, or inclusion in the diet of 0.5 per cent choline. In simple deficiency of folic acid, no change occurred in the content of pantothenic acid and coenzyme A in the liver, but excess of nicotinamide reduced both values, which effect could again be counteracted by added choline in the diet.

Mitbander and Sreenivasan (1955) reported that the content of folic acid and citrovorum factor in liver increased in rats given the narcotic, chloretone with or without folic acid. There was an increased conversion of folic acid to citrovorum factor on the days succeeding those on which chloretone was given. Chloretone depressed the xanthine, choline and tyrosine oxidases in liver which effect was reversed by folic acid.

Rege and Sreenivasan (1954) observed that in cells of *L. casei.* aureomycin in a concentration of 300 mμg./100 c.c. inhibited synthesis of pentose nucleic acid and deoxypentose nucleic acid which inhibition was countered by addition of 200 mμg. folic acid or 400 mμg. vitamin B_{12}. The authors (1954) also reported that in *S. faecalis* R. which

requires folic acid for growth, the production of deoxypentose nucleic acid varied with the folic acid content of the medium. In *L. Leichmannii* requiring vitamin B_{12} for growth, synthesis of pentose nucleic acid varied with the vitamin B_{12} content of the medium. Media not containing purines, folic acid and vitamin B_{12} singly and together showed effect on production of nucleic acids by *L. casei*. Purine degradation by resting cells of *L. casei* was inhibited to some extent in the presence of folic acid and vitamin B_{12}.

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OTHER B-VITAMINS.

Biotin and Inositol

Gothoskar and Sreenivasan (1953) studied the synthesis of biotin in Bacterium coli in a semi-synthetic medium containing a vitamin-free casein hydrolysate and found that while pimelic acid, urea or cystine added to the medium separately did not increase the synthesis, the three together did. Desthiobiotin in the medium increased the biotin synthesis to a large extent and no added cystine appeared to be required for the introduction in the biotin molecule of the sulphur atom. The authors (1953) reported that lactic and pyruvic dehydrogenases and serine-oxidase systems were partly inactivated in biotin deficient cells of L. arabinosus while the other oxidative enzymes were not affected. Biotin-deficient cells could not, unlike normal cells of L. arabinosus, dehydrogenate sodium salts of fatty acids including laurate, myristate, palmitate and stearate. B. coli cells contained a fatty acid dehydrogenase, the activity of which was less in biotin deprived cells. It was considered that biotin was indirectly acting, probably by influencing amino-acid synthesis in these enzyme systems. It was also observed (1953) that the aspartic deaminase activity of resting cells of B. coli was not affected by addition of biotin and adenylic acid or by cultivation in media with added biotin, aspartate and oleate or avidin. In cells aged at pH^4, the activity fell and was not restored by biotin and adenylic acid addition, unless glucose and phosphate were present. The aspartic oxidase activity was less in aged cells, which could not be restored by biotin and adenylic acid alone, but could be restored only to a small extent in presence of glucose and phosphate. Gothoskar, Rege and Sreenivasan (1954) reported that synthesis of pentose nucleic acids and deoxypentose-nucleic acids by L. arabinosus. L. casei and Bacillus subtilis was reduced by addition of biotin to the basal media. Aspartate, oleate and inositol which could replace biotin as growth factors for certain micro-organisms, had a similar lowering effect on the synthesis of nucleic acid.

Tirunarayanan and Sarma (1953) observed that biotin reversed growth inhibition by gammexane in a wild strain of Neurospora Crassa, while meso-inositol was without any effect. Other B-Vitamins did not affect the growth of the fungus both in the presence and absence of gammexane, In an "inositol-less" mutant of the mould, biotin in the presence of an excess amount of inositol reversed completely the growth inhibition by gammexane whereas inositol alone did so only partially. It was suggested that biotin might be concerned with the synthesis and utilization of inositol in the organism. Tirunarayanan and Sharma (1954) also reported that in Aspergillus oryzae γ-hexachlorocyclohexane

inhibited the two actions of inositol in the cell, viz. synthesis of L-amylase and riboflavin, which inhibition was countered by biotin. Folic acid, while not having any effect on these synthesis, augmented the inhibitory effect of the insecticide.

Ramachandran and Sarma (1954) reported that the reduction of α-amylase activity of plasma and of pancreas in rats fed γ-isomer of hexachlorocyclohexane could be reversed by supplements of inositol. Similarly the reduction of the amylase activity of rice-moth larvae caused by the γ-isomer could be prevented by giving them inositol. Both inositol and biotin limited the inhibiting effect of the insecticide on synthesis and secretion of α-amylase *in vitro* by slices of pigeon pancreas. Biotin had no effect on the amylase activity of the plasma or pancreas of the rats or of the larval tissue and hence the authors suggested that in the pancreas, biotin acted indirectly by stimulating inositol synthesis.

Pyridoxine

Chowdhury *et al.* (1954) reported that in phrynoderma cases, injection of 50 mg. pyridoxine twice weekly enhanced the curative effect of raw linseed oil and there was a significant increase in the iodine value of the fatty acids and in the blood cholesterol of patients given linseed oil along with injections of pyridoxine.

Pantothenic Acid

Deb *et al.* (1954) reported in pantothenic acid-deficient rats, lower ascorbic acid and cholesterol content of adrenals, lower blood sugar and liver glycogen and lower excretion of urinary 17-ketosteroids which suggested a lower adrenal cortical activity. Normal blood Na and undiminished Na and K excretion in urine suggested that adrenal hormones concerned in electrolyte metabolism were not affected in pantothenic acid deficiency.

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VITAMIN C.

In their studies on the biosynthesis of vitamin C in germinating *mung* seeds Sreenivasan and Wandrekar (1950) found no ascorbic acid oxidase on the first day of germination, but it progressively increased upto the 8th day. About 1/6th of the ascorbic acid oxidation was due to Cu content of the seed. A thermostable factor was found present in seed extracts which protected ascorbic acid against oxidation by Cu, and was active in the presence of oxidase. Germination in the dark reduced oxidase. Sohonie and Guttikar (1953) attributed the stability of ascorbic acid in *neera* from date palm (Phonix silverstres) to the presence in it of a high concentration of sulphydryl compounds and of dehydroascorbic acid reductase. Nath *et al.* (1952) found that glucose had little or no effect on the formation of vitamin C by germinating green gram seeds. Sodium acetoacetate alone had a depressing effect and together with glucose the result was similar for the first 48 hours, after which there was a big increase. 2-Tetrahydroxybutyl-5-methyl-4-carbethoxyfuran, a condensation product of glucose and ethyl acetoacetate, caused a definite increase in vitamin C formation by the germinating seeds. Nath, *et al.* (1953) reported that intramuscular injection of sodium aceto-acetate, alone or followed immediately by glucose or the condensation product of glucose and ethyl acetoacetate, caused a temporary rise in the plasma value for ascorbic acid within an hour in rats, the rise being most marked with the condensation product. Ganguli *et al.* (1954) provided evidence for the suggestion that pyruvic acid is a precursor of ascorbic acid in the rat. The incubation of liver tissue with Na pyruvate under suitable conditions was shown to produce an increase of from 15-20 per cent ascorbic acid ; in the presence of thiamine the increase was more than 30 per cent. Radio-active ascorbic acid and 2-4 dinitrophenyl-hydrazine derivative of dehydroascorbic acid were isolated by column chromatography from the urine of rats treated with chloretone and radio active pyruvamide ($CH_3{}^{14}COCONH_2$).

Roy *et al.* (1955) found that the rise in urinary excretion of vitamin C in rats receiving chloretone daily was not affected by a high intake of detoxicating agents such as glycine or by removal of the pancreas or adrenal glands. Injection of enzyme inhibitors such as malonate, iodoacetate or fluoride did not affect the urinary excretion of vitamin C in normal rats or those receiving chloretone. The tissue reserves of vitamin C in normal rats were appreciably reduced by the deficiency of vitamin B_1 or riboflavin, whereas depletion of vitamin C had no effect on the thiamine content of tissues. The urinary excretion of both citric acid and vitamin C fell to zero in vitamin B_1 deficiency and was not raised by administration of chloretone. Vitamin B_1 alone caused a rise

in citric acid excretion, but both chloretone and vitamin B_1 were necessary for a rise in urinary vitamin C. Ranganathan (1955) found that four to six weeks old pyridoxine deficient rats excreted in urine $\frac{1}{4}$ to $\frac{1}{3}$ the amount of ascorbic acid excreted by the pyridoxine-fed controls. The rise in the urinary excretion of ascorbic acid after the administration of chloretone found in normal rats, was not found in vitamin B_6—deficient ones. Sastry and Sarma (1955) reported that thiamine-deficient rat showed a very much decreased conversion of subcutaneously administered glucuronic acid to ascorbic acid as compared with the normal, showing thereby that thiamine is necessary for the proper utilization of glucuronic acid for ascorbic acid synthesis.

Rao and Giri (1952) confirmed that the inhibiting effect of ascorbic acid on urease is not due to the formation of dehydroascorbic acid, since the effect of the latter, in the pure state, on urease is negligible. Compounds like cysteine, 8-hydroxy-quinoline and Na diethyl-dithiocarbamate protected urease from inactivation by ascorbic acid, possibly by combining with traces of copper which might be present. Of six crystalline antibiotics tested by Dudani and Krishnamurti (1954) only terramycin exerted any great catalytic effect on the oxidation of ascorbic acid to dehydroascorbic acid without liberation of CO_2 at an optimum pH of 8. The activity was specific to ascorbic acid and the sample used was free from trace metals including Cu ; its activity was not affected by NaCN, glutathione, cysteine and other substances which inhibit the catalytic oxidation of ascorbic acid by Cu. Lahiri and Banerjee (1956) studied the metabolic inter-relationship between ascorbic acid and pteroylglutamic acid. Aminopterin reduced the concentration of ascorbic acid in liver, kidneys, adrenal glands and intestine ; chloretone increased it except in the adrenal glands of normal rats. Chloretone or aminopterin alone or together had no effect on the concentration of ascorbic acid in the testis and pancreas. Bagchi and Chowdhury (1954) determined ascorbic acid and Fe in serum and haemoglobin and found that the three variates were significantly correlated. The relationship between ascorbic acid and Fe was direct, whereas that of Hb and ascorbic acid was through Fe. When 500 mg. ascorbic acid daily was administered orally, there was in a majority a rise in serum ascorbic acid and Fe. This effect was not however, obtained when given intramuscularly. The authors believe that this suggests that presence of ascorbic acid in intestine is necessary for the absorption of Fe from the gastro-intestinal tract.

Banerjee and his team of workers studied in detail the effect of scurvy on the different biochemical constituents of guinea pig tissues. The amount of cholesterol and ascorbic acid in the adrenal

glands was significantly less in the scorbutic animals than in the normal ones (1951). The serum Na was decreased and K increased in all scorbutic animals. The amplitude of the contractions of a toad's heart was depressed when it was perfused with serum from the scorbutic, but not from the normal animal and the normal contractibility of the isolated uterus of a scorbutic guinea pig was depressed. There was diminished excretion of 17-ketosteroids in urine in the scorbutic animals. Thus evidence was adduced in support of the view that adrenal activity is diminished in scurvy (Banerjee and Deb, 1952). Glutathione was found to be significantly less in the blood, adrenal glands, pancreas and spleen of scorbutic guinea pigs than in the pairfeds, normal animals. Dehydroascorbic acid was present in all the tissues examined of the scorbutic animals, but not of those given ascorbic acid. It is considered that the diminished insulin excretion in scorbutic guinea pig may be due to combined effect of diminished glutathione content and increased dehydroascorbic acid content of the tissues especially of the pancreas (Banerjee *et al.* 1952). In scorbutic guinea pigs, r.b.c., p.c.v. and haemoglobin decreased and the number of reticulocytes increased. The M.C.H., M.C.V. and M.C.H.C., showed that the anaemia was of normochromic normocytic type. Leucopenia due mainly to a decrease in neutrophils was also found. Both scorbutic and normal animals were however, in positive Fe-balance (Deb and Banerjee, 1953). During the progress of survey, the capacity to acetylate p-amino-benzoic acid diminished ; the cholesterol content of the adrenal glands, spleen and lungs fell, that of the testes and small intestine increased significantly ; the liver and kidney showed no change. It was suggested that the disturbance of cholesterol metabolism in the guinea pig deprived of vitamin C may be responsible for the lowered function of adrenal gland (Belavady and Banerjee, 1954). The hexokinase activity of skeletal muscle and liver from scorbutic guinea pigs was significantly less than from pair-fed normal animals. In the kidneys it was greater and in the pancreas there was no difference (Banerjee and Ghosh, 1955). Coenzyme A activity in the liver, kidneys and brain was unchanged and total body cholesterol rose. It is suggested that oxidation of acetate by way of the tricarboxylic acid cycle is inhibited in scurvy and that excess of acetate might be used to increase synthesis of cholesterol (Lahiri and Banerjee, 1956).

Banerjee *et al.* (1953) found that intravenous injections of 1 or 1·5 g. dehydroascorbic acid per kg. in rabbits caused profuse salivation and lachrymation, dilatation of the pupil and respiratory failure, but contrary to the finding of Patterson (*J. Biol-Chem*,, **183**, 81, 1950) did not cause a persistent high blood sugar level or persistent

diabetic type of glucose tolerance curve. The initial rise in blood sugar could be prevented by a prior injection of dihydroergotamine methanesulphonate. The rabbits did not excrete sugar in urine or show any histological change in the pancreas, adrenals or pituitary glands. Banerjee and Belavady (1953) and Chakravarti and Banerjee (1955) compared the proportion of ascorbic acid and dehydroascorbic acid in blood of normals and in persons suffering from infections. In various infectious diseases like tetanus, typhoid, tuberculous meningitis etc., the concentration of dehydroascorbic acid in blood was greater than normal and ascorbic acid was correspondingly lower. Values for dehydroascorbic acid were greatest in those patients suffering from the infectious diseases, who ultimately died. During convalescence, dehydroascorbic acid decreased and ascorbic acid increased. The authors (1955) suggest that for prognostic purposes, dehydroascorbic acids should be estimated in all patients with acute infection. Rowlands *et al.* (1955) determined the total ascorbic acid concentration in whole blood from 43 normal adults, 100 out-patients with gastric disturbances and 100 miscellaneous patients. The normal value was 0·88 mg./100 ml. and the values were lower by 50 per cent in the other two groups. Ascorbic acid saturation tests were made on 43 normals. There was good correlation between the number of days required to reach saturation the kind of diet and the initial blood value for ascorbic acid. It was therefore, concluded that saturation tests provided little additional information and that the dietary history and blood ascorbic acid level were sufficient for survey work.

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VITAMIN D

The mode of action of vitamin D has been a subject of detailed study at the Nutrition Research Laboratories, Coonoor. Dikshit and Patwardhan (1952) found that vitamin D incorporated in calcifying media in a fairly dispersed condition showed no local action on the bones. Rachitic bones from animals previously administered with vitamin D, but not exhibiting any *in vivo* calcification, showed much greater calcification *in vitro* than the bones of animals not given vitamin D. This *in vitro* calcifying capacity of the rachitic bones appeared to increase with the increase in time between vitamin D administration and killing of the animal for removal of the bones. The maximum calcifying capacity *in vitro* coincided with the first evidence of calcification having commenced *in vivo*. The authors suggested that calcification depended not so much on the composition of the surrounding medium as on the state of cartilage cell and matrix and that vitamin D probably influenced the metabolism or structure of the epiphyseal cartilage cell and the relative matrix rendering them capable of laying down the bone salt. Tulpule and Patwardhan (1954) found that for rats in the early stages of rickets, the anaerobic glycolytic activity of the epiphyseal cartilage was considerably higher than for those given vitamin D. Later the difference disappeared and it was not as a whole statistically significant. The aerobic oxidation of pyruvate on the other hand, diminished steadily in rachitic cartilage and after 20 days on the rachitogenic diet seemed to have ceased. Tests with liver and kidney homogenates showed no loss of capacity to oxidise pyruvate by rachitic rats. The authors suggested that failure to oxidise pyruvate interfered with the supply of citrate to the bone and hence significance of citrate in rickets. In their histochemical study of rachitic epiphyseal cartilage during healing in the rat, Ramalingaswami *et al.* (1954) found that administration of vitamin D brought about a marked increase of glycogen in the proliferative zone of rachitic cartilage of rat tibiae in a majority of animals before the onset of calcification, followed by a reduction in all animals at its commencement. While the cartilage matrix of untreated rachitic rats showed a uniform pyroninophilia, the matrix in and around calcifying areas in the treated rats showed marked reduction and alteration in pyroninophilia. The reaction for alkaline phosphatase was usually reduced in and around sites of calcification. A similar sequence of changes was observed in rachitic cartilage undergoing calcification under the influence of a citric acid : Na citrate mixture. It was suggested that glycogen in cartilage cells and the glycoprotein in the martix were intimately connected with calcification. The failure of rachitic cartilage to calcify could be due to a disturbance in glycogen metabolism, the orderly progress of which might be essential for the formation of an approximate matrix.

Dikshit (1955) reported that removal of parathyroid glands had no significant effect on the bone ash content of the femur and the width of the epiphyseal cartilage in the tibia in rats maintained on a rachitogenic diet low in P with or without oral feeding of vitamin D. Further, parathyroidectomy in rats given subcutaneously massive doses of vitamin D did not afford any protection against the toxic symptoms of hypervitaminosis D. Dikshit concluded that the action of vitamin D is not mediated through the parathyroid glands. Joshi and Dikshit (1956) found that the total P of rachitic epiphyseal cartilage started increasing after a single oral dose of 4000 I.U. vitamin D and reached a maximum at 96 hours coinciding with the maximum increase in the calcifying capacity as shown by the cartilage *in vitro* and with the onset of demonstrable calcification *in vivo* after similar treatment.

Chitre and Damle (1956) studied the effect of high doses of vitamin D on N excretion in rats. Administration of vitamin D 20,000 I.U. daily, resulted in an increase in the urinary output of all forms of N and the N-balance became negative. Signs of toxicity then became severe, with loss of weight, increased output of Ca and P and reduced food intake. Rats getting excess vitamin D and on a protein free regimen died earlier than the animals which received protein. They concluded that in vitamin D excess there was no damage to the liver and that it was exogenous protein metabolism that was primarily affected.

Laha *et al.* (1953) reported that in 6, out of 10 men, ("patients chosen at random from the wards") 24 hours after intramuscular injection of a massive dose of 15 mg. calciferol, serum Ca was significantly reduced and serum inorganic P was significantly raised : one subject showed a marked fall in serum P and the others no significant changes.

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ENZYMES

Giri and coworkers (1951, 1951*a*, 1952) have separated and identified the enzymes analysed, phosphatases and phosphorylases by paper chromatography using aqueous solutions of acetone, ethanol and sodium chloride as developing solvents. The presence of lipase in human blood plasma has been demonstrated by Srinivasan and Patwardhan (1952) by taking advantage of the inhibitory action of sodium tauroglycocholate on esterase—tributyrin system. Dhungat and Sreenivasan (1952) have studied the synthesis of xanthine oxidase by rat liver slices *in vitro*. The xanthine oxidase activity of the liver slices from protein-depleted rats was increased by aerobic incubation with serum containing glucose and still further increased by supplementing the serum with glycine, methionine, riboflavin and bicarbonate. The increase in enzyme activity was associated with an increase in flavin-adenine—dinucleotide and also with synthesis of the protein component of the enzyme. The authors also demonstrated that livers from folic acid deficient rats were capable of synthesising xanthine oxidase *in vitro* whereas livers from normal rats were not. The same authors (1954) have described an improved technique for the manometric estimation of xanthine oxidase in rat tissue homogenates, using pyrophosphate buffer at pH 8·6 and thereby eliminating endogenous respiration without affecting the enzyme activity

De (1955) found that thiamine hydrochloride at a concentration of $7{\cdot}6 \times 10^{-3}$M. completely inhibited cholinesterase in human serum directly without affecting the substrate. The degree of inhibition was lineally related to the concentration of thiamine between $7{\cdot}5 \times 10^{-4}$M and $7{\cdot}5 \times 10^{-3}$M.

Deolalkar and Sohonie (1954) reported that fresh-water fish contained a thiaminase with optimum activity at pH 7·0 whereas the thiaminases of fish from brackish or salt water had optimum activity at pH 3·6 and pH 5·6 respectively. The water extracts from the different varieties of fish, lost activity after dialysis, but addition of boiled original extract, restored activity which suggested the presence of a coenzyme. Addition of salts of manganese and cobalt restored the activity of the enzyme exhibiting optimum activity at pH 3·6 and 5·6, but not that at pH 7·0. The three enzymes could not be separated by fractional precipitation with organic solvents or by absorption-elution procedures tried.

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ENERGY METABOLISM

Ramaswamy and Mookerjee (1953) concluded from the surface area measurements made by the Du Bois' tape method on 18 healthy Indian males ranging in height from 5 feet to 5 feet 11½ inches and in weight from 90 to 158 lb. that the Du Bois' height-weight formula was applicable to Indians although it was derived from studies on American whites. Later Banerjee and Sen (1955) determined the surface area in 15 male subjects of various parts of the body also by the tape method coupled with a plaster/mould for the head and face. They found the observed surface area to be larger by 1·97 to 5·80 per cent than that calculated from Du Bois' height-weight or linear formula. The authors have suggested a new constant ($C=74{\cdot}66\pm0{\cdot}23$) in the Du Bois' Height-Weight formula for use with Indian subjects.

Mahadeva (1954) measured in Edinburgh the BMR in 20 healthy South Asiatic men aged between 20 to 41 years after residence in Scotland for periods of from 1 month to 4 years. Comparing the results with those obtained on an equal number of healthy Europeans, he found no significant difference in BMR between the two groups. The value for the Asiatics was 40·1 and for the Europeans 40·6 cal./sq. m./hr. Gopalan *et al* (1955) in their study on BMR and body composition in normal subjects found that the BMR expressed per square meter body surface varied between 32·3 and 40·5 cal./hour whereas expressed in terms of cell solids, it varied between 20·1 and 22·9 c.c. O_2/kg.cell solids/minute. The authors found that the correlation with cell solids was higher than with the surface area and have suggested cell solids as a metabolic reference standard.

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DIGESTION

Pathak and co-workers (1952, 1953 and 1954) have studied in human subjects the gastric digestion and evacuation time of various types of Indian foods. Milk or milk preparations like curds, cheese, butter, *shrikhand*, *basundi* etc., were given to healthy young adults in 100 g. portions and the gastric response studied. The aspirated material was examined for consistency of food particles, colour, mucous, bile, free and total acidity, for presence of peptones and starch and reducing substances. Similar studies were done with some common preparations of cereals, pulses, and groundnuts and with foods prepared from egg, fish and meat.

The vegetarian foods left the stomach in $2\frac{1}{2}$ to $3\frac{1}{2}$ hours and the liquefaction of the food commenced usually in 2 hours. Boiled milk, with and without sugar and ice-cream left the stomach within 3 hours, curds and butter a little later and *Shrikhand*, *Basundi* and cheese took over 4 hours. The gastric evacuation time for fish, chicken and meat dishes was over 4 hours. A single boiled or raw egg was evacuated in little less than 3 hours and three eggs in about $4\frac{1}{2}$ hours.

Acid response with some of the vegetarian articles, especially those rich in protein, was high, but not as high as with similar quantities of some milk preparations. Boiled milk with and without sugar and ice-cream evoked less secretion of acid than the other milk preparations. Free and total acidity on eggs, raw or boiled, was higher than on alcohol meal. Fish, chicken and meat produced more acid secretion than eggs or some of the milk preparations. Starch when present in protein rich foods diminished the acid secretion.

Protein digestion began in about 15 minutes after the administration of food. However, it was very feeble in that even as small an amount as 3 g. protein in 100 g. portion was not fully converted into peptones until the food left the stomach.

Pathak and Pai (1956) also carried out fractional gastric analysis with alcohol meal on 50 normal male medical students 18 to 24 years old. The stomach was emptied within 3 hours in 70 per cent cases and in 90 per cent at the end of $3\frac{1}{2}$ hours after giving the test-meal. The average volume of fasting juice was found to be 37·2 c.c. The highest total acidity was on an average $32{\cdot}55 \pm 2{\cdot}76$ c.c. N/10 acid per cent and was reached after 1 hour and more or less retained upto $2\frac{1}{2}$ hours. 8 per cent of the subjects showed no free acid (histamine test was not done) and 18 per cent gave a hyperchlorhydric response.

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DIET AND NUTRITION SURVEYS

Diet Survey:

The dietary habits and the nutritional status of the various sections of the Indian population have been studied by many workers. Sen Gupta (1952) observed that in the Abor Hills area of the North Eastern frontier of India, inhabited by aboriginal tribes, milk and its products, fats and oils, and sugar and jaggery were unknown. Only 63 per cent of the families in the "higher" economic group (those self-sufficient in grain) and 40 per cent in the rest were receiving 3,000 calories per consumption unit daily. Their intakes of protein, calcium, vitamin B_1 and nicotinic acid were satisfactory but those of vitamins A & C and riboflavin were inadequate. Lal (1950 and 1953) found that the depressed classes of Bihar, Chairo and Dusadh had inadequate intakes of all nutrients whereas Nomia and Chamar had adequate energy intake but lower intakes of calcium and vitamin A.

In a survey of the Dangs District of Bombay State, Radhakrishna Rao (1954) found that the Dangis, an adibasi tribe inhabiting the area, lived on a diet which was inadequate in quantity, made up mainly of cereals and starchy tubers and almost totally free from cooking oils and animal foods. Intake of milk and milk products and fresh vegetables and fruits was also found to be negligible. The diet lacked in proteins, fats, vitamin A and vitamin C. Inspite of negligible intake of milk, the calcium intake of the diet was high because of the millet, Ragi or Nagli which is the staple food for the greater part of the year in the area. In school children generalised undernourishment, stunted growth and marked deficiency of vitamin A were evident. The common major ailments amongst the adults were Malaria and other infective fevers, conjunctivitis, non-specific diarrhoeas, tropical ulcer etc. The incidence of nutritional disorders was in general insignificant. There was no evidence of helminthic infection as revealed by examination of stool samples. Haematological examination indicated that secondary anaemia due mainly to iron deficiency was present in most of the subjects.

Families of the industrial workers in the cities of Bombay and Calcutta have been surveyed for their dietary intakes. DeMello *et al* (1950) found that in the 29 families of factory workers in Bombay the average intake of animal protein and vitamins A, B_1 and C were low. Anaemia was found in 63 out of 102 persons and in 39 of these, there was parasitic or protozoal infestation of the gastrointestinal tract. The diet survey conducted by Mayer *et al* (1956) in 213 male employees in a Jute Mill in Calcutta showed that intakes of total protein and of vitamin B_1, niacin and C were adequate; riboflavin intake was low. The energy

intake was not related to income and was lowest in the light work group and was equally high in the sedentary, heavy and very heavy work groups.

Studying the nutritional level of students and nurses of Assam Medical College, Banerjee (1953) reported that both male and female students were having satisfactory diets except for Fe and for vitamins A, B_1 and riboflavin the intake of which was low or marginal. Nurses' diets were both inadequate and ill-balanced. Milk, and its products, fresh fruits and vegetables were little used by any group. Sarkar and Sarkar (1953) calculated the average nutritive value of the diet of 165 hospital nurses in Burdwan, W. Bengal by weighing the raw food at intervals for a year and found that the intakes of all the nutrients excepting Fe and nicotinic acid fell substantially short of the recommended levels. Govil *et al* (1953) computed the mean daily intakes of nutrients per head by weighing for one week the food consumed by school boys in 14 hostels in U.P. and found that compared with the recommended allowances for Indian children, the diets were low in animal protein, Ca and vitamin A, ascorbic acid and riboflavin intakes being on the borderline. Subrahmanyeswara Rao (1955) on examination of more than 9000 students in two colleges in Masulipatam in Andhra Pradesh over a number of years from 1949, reported that the energy intake of 2400 cal. as calculated from estimates of food consumption, fell short of requirement taken as 2600 cal. Defective sight and pyorrhoea were the commonest clinical abnormalities found.

Radhakrishona Rao (1956) reported results of diet surveys carried out during the five years 1945-50 in 454 messes attached mostly to college and school hostels, spread over 19 districts of the then Bombay Province. The diets were found to be generally deficient in milk, fresh vegetables, fruits and fleshy foods. In the institutions other than school and college hostels which have been classified to be in the lower economic group, the diets were inadequate both in quantity and quality whereas in the well-to-do group of school and college messes, the diets though sufficient in quantity was poor in quality. The districts of Gujarat showed higher consumption of milk than other districts. The dietary fat intake was generally low in all but college hostel messes.

From a statistical study of the data of the Family Budget Enquiry done in 1950-51 by the West Bengal Government, Chakravarti and Bandyopadhyay (1953) found that the consumption levels of cereals by adult males and females and children in a family varied according to the total income. In the higher income group women and children got a higher share of cereals than in the low income groups. From a study of the diet survey on 238 U.P. families done in 1952, 1953 and 1954 Govil

et al (1956) observed that as income increased, wheat replaced rice and cheap grains and ghee replaced cheaper vegetable oils; more vegetables, fruits and milk were used. Nearly half the families took meat and other non-vegetarian foods, but intakes were irregular and did not appear to depend upon income. The monthly cost of a balanced diet in U.P. was calculated to be Rs. 34/- including fuel. Hence it was suggested that while people with incomes of less than Rs. 40/- p.m. could not afford it, the higher income groups should be educated to use more of green vegetables, milk and animal protein. As part of the national sample survey, the Indian Statistical Institute (1954) have reported consumer expenditure data collected from 6,770 households of which 5,080 were scattered over 905 villages and 1,180 in 49 towns and 510 in the 4 cities of Calcutta, Bombay, Delhi and Madras. In the rural areas, mean consumption values per household per week for all items was Rs. 28·62 of which Rs. 19·08 was for food items, whereas the corresponding values for the urban areas were Rs. 37·12 and 19·92

A diet survey by Lal (1952) during August, October, 1950 in two districts of Bihar where the maize and paddy crops had been destroyed by floods and the landless labourers were, consequently underemployed showed that in general, consumption was poor, consisting mainly of rice and maize with small amounts of vegetables. The middle class families had fish and the labourers snails, crabs and oysters, but only in small amounts. Milk was a luxury, though much limited, of the middle class only and fat intake was low. In the scarcity districts the energy intake of the labourers was about 1,500 Cal./ consumption unit daily and that of the middle class barely 1,900 Cal. compared with the labourers in the unaffected district who had 2,250 Cal. Nutrition survey showed that the children of the middle class in the scarcity area were comparable to the labourers' children in the unaffected area.

Lal (1952) repeated in 1949 and 1950 the diet and nutrition surveys in the aboriginal tribe of Hos originally done by Mitra (Mitra, K., *Indian J. Med. Res.*, **30**, 91, 1942) in 1940-41 and found that the income of rural families had risen by 200 to 600 per cent over that recorded in 1940-41; consumption of foodstuffs had also gone up. The diets were adequate in 1949-50 except for fat and vitamin A. There was positive correlation between income and consumption level. A similar repeat survey (Lal, 1954) in 1951-52 of the aboriginals, Santhals, Sauriapaharis and Malpaharis surveyed in 1938-39 by Mitra showed that though family income had risen in the later years, increases in consumption were confined to cereals and pulses with a decrease in green vegetables. Cereals provided 92 per cent of the total energy in the diet and intakes of all nutrients were below the recommendation of N.A.C. of the Indian Council of

Medical Research. Boys were found to be taller after 7 years and girls after 9 years than children surveyed in the previous survey and as a whole a better nutritional status was found in the latter survey. The incidence of nutritional diseases was lower in Santhal than in Pahari children. Lal (1950) reported that in the mining population in Jharia Coal-fields, though the family income in 1947-48 had risen by 314 per cent, over that in 1939-40 as found by Mitra the intake of nutrients was lowered. Rice intake decreased in the lower-income group and increased in higher-income group whereas pulses and non-leafy vegetables had increased in all, and leafy vegetables, fat oils and milk had decreased in all the groups. The average energy intake and intakes of minerals and vitamins had decreased in all. The incidence of xerophthalmia, angular stomatitis and caries was higher and also the total number of children affected by one or other of the diseases. Comparison of results of diet surveys done in 1943 and 1951 in Darbhanga district of Bihar (Lal, 1953) showed a considerable lowering of the intakes of foods, nutrients and calories, excepting for Ca intake. People in the food scarcity area suffered from malnutrition, underweight and some with oedema in legs.

Govil and Mitra (1954) reported that evaluation of individual diets in terms of the proximate composition by calculation from food tables and by chemical analysis showed such wide differences in Ca, P and Fe that calculated nutritive value of individual diets would be of doubtful value. The differences in the value for Fe was however, considered to have no practical significance.

Nutrition Survey :

In a nutrition survey of 4,191 children from 44 tea estates in the Brahmaputra Valley of Assam during the years 1948 and 1949, Gilroy (1951) found that xerophthalmia was very common, dry skin moderately common and phrynoderma absent. In a sample of 250 children no striking reduction of xerophthalmia was achieved by the daily addition to the usual diet of 3,000 I.U. of vitamin A for 7 months, though the severity of the condition decreased. The fact that phrynoderma was not found, though the vitamin A intake was inadequate proved once again the non-relationship of vitamin A to the deficiency state. There was complete absence of the major deficiency diseases, beri-beri, scurvy and rickets. Results of diet and nutritional survey in families in Sirur in Poona District and community project area in Kolaba District of State showed that the average diets were unsatisfactory being low in protective foods and in fat. The observation that the intake of protective foods with rise in economic status was confirmed. Nutritional deficiency was mainly due to a deficient intake of fat and vitamin A in both areas. Govil (1952) in a

clinical assessment of the nutritional status of 6,371 school boys 90 per cent of them being from rural areas and between the age range 5 to 16 years reported that growth was greatest between 13 and 15 years; 59·3 per cent of them were affected by one or more of the deficiency conditions. The incidence of vitamin A deficiency was common, the incidence of deficiency of other vitamins increased in the groups with poorer physique. Only 18·3 per cent of the boys had dental caries. Singh (1955) found that of 46,650 children who attended the hospital out-patient department in the three years, 1952 to 1954, 5 per cent showed xerosis conjunctivae and 1·5 per cent had clinical rickets. Out of 3,239 children admitted as in-patients, 0·6 per cent had rickets and 0·4 per cent scurvy. It was noted that although a large proportion of the child population is malnourished, only a small proportion develop gross anatomical changes requiring treatment at hospital.

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1. Process of manufacture.
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Indian Food Packer, 7, No. 7 and No. 8.

GROWTH AND DEVELOPMENT

In recent years not much of attention has been paid to a systematic study of the growth and development of Indian children. It is difficult to arrive at an authentic picture for the country as a whole, only from the few reports available, in that no attention has so far been given to the selection of adequate number of samples and to the sampling method itself so as to cover a representative cross-section of the Indian population.

Rao and Bhattacharjee (1952) have followed the gain in weight during the first month, based on daily weighings of 26 male and 20 female full-term, breast-fed infants of middle class rice-eating parents from Bengal or Madras and residing in Calcutta. Only infants weighing 4 lb. 10 oz. and above were included in the study. Mean birth-weights were 6 lb. 12 oz. for boys and 6 lb. 8 oz. for girls. The same authors (1953) have constructed growth curves from the values of mean weights and lengths measured weekly from birth to 1 year in 43 breast-fed middle class infants.

The effect of vitamin B_{12} and antibiotics on the possible augmentation of growth in under-nourished Indian children has been studied. Someswara Rao *et al* (1955) in two trials made with 26 destitute Indian girls, once when the diet consumed was grossly inadequate in energy value and protein as well as in Ca, vitamin A and vitamin B complex and once again after 12 months of an improved diet, found that oral supplementation of 25 μg. vitamin B_{12} daily for 14 to 15 weeks, could not produce any demonstrable effect on growth and N-retention. Banerji (1956) studied the effect of aureomycin in 100 under-weight Indian children in a residential institution and found that the antibiotic fed at a level of 1 mg./kg. body weight daily for 24 weeks, did not improve growth.

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BLOOD

A. Haematological studies in normals :

Haematological values for the apparently healthy in different sections of the Indian population as determined by several workers during the period under review are given in the Table (p. 92). Besides these, Someswara Rao *et al* (1954) in a large scale haemoglobin survey of more than 2,000 boys and girls in the district of Nilgiris, at each age-level from 2 years onwards upto 19 years in boys and upto 13 years in girls, found that for each age group the haemoglobin was 1 to 2 g. lower than the British MRC averages.

Physiological variations due to environmental conditions :

Altitude has been found to affect the normal haematological values in humans. The haematological response of a group of Indian troops at moderately high altitude in extreme cold environment was studied by Mookerjee and Ghose (1951) subjects acclimatised to altitudes between 6,000 and 10,000 ft. serving as controls. An increase in the r. b. c. on the 10th day of acclimatisation as also increases in p. c. v. and m. c. v. were found. But there was no similar increase in the haemoglobin values until after the 50th day of acclimatisation. Examinations of blood of Indians at high altitudes showed no increase in the reticulocyte count and hence Das Gupta (1952) does not support the hypothesis of Hurtado *et al* (*Arch. Int. Med.* **75**, 284, 1945) that the increases found in r. b. c. and Hb as altitude increases are the results first of a release of stored blood and later of greater erythropoietic activity.

Blood and plasma volume in healthy Indians :

Chaudhuri *et al* (1951) reported that blood and plasma volume determinations in apparently healthy Indians of both sexes by the Evans Blue method, showed a high degree of correlation with body weight and body surface. Mean blood volume was found to be 9 per cent and mean plasma volume 5 per cent of the body weight. The results are given in the Table below :

TABLE

Ref. No.	No. of observations		Blood volume		Plasma volume		Total circulation	
	M	F	ml/kg. body wt.	ml/m² body surface	ml/kg. body wt.	ml/m² body surface	Protein in g.	Hb g.
10	41	9	83·10	2770	47·70	1591	168·5±32·5	580·3±146·2
11	115	..	84·58	..	48·61	..		
..	..	39	83·26	..	51·48	..		

BLOOD

Haematological studies in normals.

Ref. No.	Place	Particulars	No.	Age in years	Hb. in g.% ±S.E.	r.b.c. in millions/cmm ±S.E.	p.c.v. in % ±S.E.
1.	Howrah	**New-born infants**					
		Cord Blood	26	..	14·43±0·457	5·43±0·166	..
		Heel Blood	23	..	15·52±0·180	6·52±0·046	..
2.	Poona	**School children of advance communities**					
		Male	100	6 to 12	12·95±0·096	4·99±0·038	..
3.	Punjab	Punjabi females, (medical students and nurses)	100	17 to 24	13·09±0·086	4·55±0·004	41·6±0·270
4.	Assam	**Medical Students**					
		Males	73	-do-	14·76±0·131	5·20±0·105	44·2±0·515
		Females	12	-do-	12·65±0·225	4·20±0·139	39·5±0·636
		Nurses	38	-do-	12·10±0·244	4·08±0·078	39·1±0·649

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B. Studies in Blood Chemistry in Health.

The average values of the different constituents of blood in health have been determined by several workers. In Table (p. 96) are given the average values of the blood constituents in healthy Indians. Apart from the data presented in the Table, further available information on the blood constituents is summarised below,

Plasma proteins :

As shown in the Table, the normal values for plasma proteins vary widely, as also those for the different protein fractions. This is mainly attributable to the different methods of estimation of total protein as well as of fractionation employed by different workers.

Of the many factors affecting the amount and the pattern of plasma proteins, the effect of age, sex, vegetarianism and feeding of antibiotics have been studied. Chakravarti and Mittal (1951) in their studies in Agra, found that the value for plasma protein was highest in the lowest age group and was higher in men than in women and in non-vegetarians than in vegetarians. Globulin and fibrinogen tended to increase with age and were higher in vegetarians. Blood urea was slightly higher in women and in vegetarians and increased with age. Satoskar and Lewis (1954) and Dhopeshwarkar *et al* (1956) have also reported difference in the plasma protein pattern in vegetarians and non-vegetarians. The latter authors reported that albumin was lower and globulin higher, particularly γ-globulin, in vegetarians and suggested that high globulin may be a compensating factor, probably resulting from consumption of proteins which are globulin forming (Dhopeshwarkar, *et al* 1956).

Satoskar and Lewis (1954 and 1955) reported that oral feeding to groups of students of 50 mg. terramycin or tetracycline daily for two months produced a rise in their plasma albumin and a fall in globulin. The authors attribute the changes to the effect of antibiotics on intestinal bacterial flora which, they suggest, indirectly influence protein metabolism.

Plasma amino acids :

Iyer (1955) has described a paper chromatographic method for the quantitative estimation of free amino acids in human plasma. In a study of 50 cases of hypochromic anaemia Iyer (1956) found significantly higher levels of plasma glycine in anaemia compared to those in normals. The author suggests that the increased concentration in plasma of glycine, which is an essential building block in the biosynthesis of haemoglobin,

may reflect an attempt on the part of the body to make good the deficiency of haemoglobin, even though the deficiency is not in the supply of glycine itself, but in other factors. Alternately the author suggests that the production of glycine may be proceeding at an essentially normal rate, but its utilization may be subnormal resulting in an increase in plasma concentration. Iyer (1956) also studied the distribution of glutamine, glutamic acid and aspartic acid between erythrocytes and plasma. Aspartic acid was found only in traces in many plasma samples. Red cells contained much more aspartic acid and glutamic acid than plasma, but glutamine was almost equally distributed between the two.

Serum calcium, inorganic phosphorus and alkaline phosphatase :

Chitre *et al* (1954) in their study of the sera of more than 500 Indian children, found that the serum Ca and inorganic P did not show significant variations due to age, while the alkaline phosphatase activity was lowered as the age advanced; it was 12·5 ± 1·081 Bodansky Units in children below 1 year old and gradually diminished to 5·8 ± 1·018 units in children 12 years old. Chakrabarty and Sen (1953) found a considerable rise in serum alkaline phosphatase in all conditions of severe liver damage as well as in cases of bone tumours, rickets and adolescent rickets.

Blood in pregnancy :

Observing the blood picture during normal pregnancy in middle class Bengali women, Ganguli (1954) found that haemoglobin and r. b. c. were lower in pregnant women than in non-pregnant women whereas the total w. b. c. and erythrocyte sedimentation rate were raised in pregnancy and increased as pregnancy advanced. The average r. b. c. was about 1 million less than in non-pregnant controls and Hb, 10 per cent lower.

Mukerjee (1953) reported that the total protein concentration of the plasma fell during normal pregnancy, mainly, due to a decrease in the albumin concentration, the lowest value being attained in the ninth month of gestation. The globulin concentration altered only to a slight extent and the fibrinogen content of the plasma increased and reached a maximum level at term. Ghosh (1951 and 1952) reported a significant fall in serum calcum in pregnancy, the fall beginning early in pregnancy, the serum calcium reached the lowest value at the ninth month. The author suggested that if correction was made for the rise in blood and plasma volumes serum Ca showed actual increase with advance in pregnancy. The author also found that corrected for haemodilution there was an absolute increase in serum phosphorus, except during the sixth month.

Table

Constituent of blood in normal Indians

Serial No.	Particulars	Number of males	Number of females	Total No. of subjects	Age group in years.	Range of values	Ref. No.
1.	Total protein in g. %	40	..	40	20—38	6·17— 7·84	1
		75	..	75	25—45	6·20— 8·00	2
		45	30	75	..	5·33— 8·22	3
		40	..	40	22—40	6·87— 8·45	4
		65	35	100	10—60	5·44— 8·10	12
2.	Albumin in g. %	40	..	40	20—38	4·17— 5·27	1
		75	..	75	25—45	3·90— 5·50	2
		45	30	75	..	3·01— 4·81	3
		40	..	40	22—40	3·77— 5·72	4
3.	Globulin in g. %	40	..	40	20—38	1·79— 2·85	1
		75	..	75	25—45	2·00— 3·30	2
		45	30	75	..	1·50— 3·30	3
		40	..	40	22—40	2·09— 3·89	4
4.	Albumin : globulin average	..	..	230	..	1·50— 2·08	1,2,3,4
5.	Fibrinogen	45	30	75	..	0·17— 0·52	3
6.	N.P.N. in mg. %	65	35	100	10—60	18·0 — 45·0	12
7.	Urea N in mg. %	65	35	100	10—60	20·0 — 50·0	12
8.	Uric acid in mg. %	..	..	16	..	1·3 — 2·8	15
9.	Sugar mg. %	..	..	36	..	70·0—110·0	15
10.	Reduced glutathione mg. %	..	..	36	..	22·0— 51·3	15.

11.	Total cholesterol mg. %	30	..	30	20—30	155·2 —190·2	13
12.	Free cholesterol mg. %	30	..	30	20—30	47·1 — 65·2	13
13.	Ester cholesterol mg. %	30	..	30	20—30	99·0 —143·0	13
14.	Lipid P mg. %	20	..	20	20—30	9·8 — 12·0	14
15.	Sodium mg. %	15	17	32	18—46	320·0 —335·0	16
16.	Potassium mg. %	15	17	32	18—46	16·9 — 21·1	16
17.	Calcium mg. %	150	50	200	18—40	9·2 — 12·8	17
		..	..	545	0—12	8·5 ± 0·77 — 10·8 ± 0·69	18
18.	Inorganic P mg. %	150	50	200	18—40	3·0 — 5·0	17
		..	..	545	0—12	4·7 ± 0·122— 6·2 ± 0·419	18
19.	Vitamin A in I.U./100 ml.	40	38	78	..	25—165	20
20.	β-carotene in/μg./100 ml.	40	38	78	..	67—583	20
21.	Tocopherol in mg/100 ml.	..	..	42	..	0·98 — 1·88	24
22.	Bilirubin in mg/100 ml.	..	..	100	..	0·10 — 1·0	26

Studying the lipid metabolism in pregnancy, Ghosh (1952) found a steady increase to peak values during the first half of pregnancy in total and ester cholesterol ; free cholesterol fell steadily during the period and thereafter rose to the initial value by the 32nd week and this was maintained to term. During the second half of pregnancy, ester cholesterol showed a fall until the 36th week, with a subsequent rise to term. Total cholesterol remained high, but like the ester, showed a fall during the 32nd to the 36th week and a subsequent rise to term.

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ANAEMIA

Anaemia, especially due to nutritional defects is widely prevalent in India. In recent years, different workers have paid particular attention to the study of the pattern of anaemias in infants, children and adults in different parts of the country.

THE TYPES OF ANAEMIAS

An investigation by Pohowalla and Rangam (1955) on 100 infants less than two years old and with less than 10 g. Hb/100 c.c. blood showed that the anaemia of infants in Central India was normocytic and hypochromic associated with iron deficiency. Tuberculosis has been found to have an important contributory influence of infant anaemias in India. A study by Chaudhuri (1951) on 30 children 1 to 12 years old, coming from the poorest classes and consuming diets very low in protein, revealed severe or moderately severe anaemia which was often hypochromic, though one case of megaloblastic anaemia which responded to vitamin B_{12} was also found. The total plasma proteins and albumin were much lower than in normals. In 9 cases oral feeding of protein hydrolysate for two weeks produced immediate clinical and haematological improvement and an increase in the albumin fraction. The remaining anaemic children improved on a diet containing 2 to 2·5 g. protein daily/lb. body weight and treatment with iron preparation and blood transfusions when required.

Jhatakia and Damany (1954) in a study on 62 males and 38 females in Bombay, 10 to 72 years old, found that 72 of the cases were due to nutritional defects; 35 per cent of the cases were microcytic and 60 per cent macrocytic. Bone-marrow examinations revealed that only one was megaloblastic. The existence of macrocytic anaemia with non-megaloblastic marrow may, it is suggested, be associated with a low level of blood plasma proteins.

A study of the case-records of Lady Hardinge Medical College Hospital, New Delhi, showed that anaemia cases in Delhi women showed a rise from 1932 reaching the peak in 1944 with an incidence of 16 per cent of all the cases and coming down to 9 per cent in 1950. Chaudhuri (1951) reported work on 140 adult females from poor and middle classes with poor dietary history. Average Hb in them was 4·56 g per cent and plasma protein 4·68 g. per cent; 18 of the cases were reported to have shown marked improvement in Hb and plasma protein on oral treatment with protein hydrolysate for one week. 18 of the 20 nutritional megaloblastic anaemia cases responded to vitamin B_{12} therapy. In yet another study, the same author (1955) treated in groups, 60 patients with

macrocytic, normocytic or microcytic, normochromic or hypochromic anaemia with milk powder, milk or meat protein hydrolysates, hydrolysates of mixed animal and vegetable protein or a groundnut protein preparation. Treatment with animal protein preparations caused significant improvement in values for plasma protein, Hb, r, b. c. and p. c. v. and in the clinical condition.

Iron deficiency anaemia :

A satisfactory haematological response to intravenous iron was reported by Mathur (1951) Jhatakia (1951), Dhariwal and Mehta (1951) and Deshmukh (1952) in cases of microcytic hypochromic anaemia. The last named author, found that intravenous iron therapy with saccharated iron oxide was a safe and rapidly effective method of increasing the Hb content as well as ameliorating the general clinical condition of the patient (1952). Twenty five anaemic cases judged to require Fe, were treated by Jhatakia (1956) with a combination of iron and cobalt intravenously. No untoward side reactions were found due to the intravenous therapy with cobalt. The response to Fe and Co was better than that to Fe alone.

Nutritional macrocytic anaemia :

Das Gupta *et al* (1953) treated 22 cases of nutritional macrocytic anaemia, 18 to 60 years of age with vitamin B_{12}. Initially 7 cases were treated orally with 100 to 500μg. of vitamin B_{12} out of which in only one case with 90μg. a good response was obtained. In two cases fair response was obtained with 40 to 45μg. and in four cases there was no appreciable improvement which later on responded very well to parenteral vitamin B_{12}. In parenteral therapy given intramuscularly in graded doses from 30 to 600μg. though the initial response was good in 14 cases, vitamin B_{12} alone could sustain improvement only in 5 cases. Folic acid at this stage showed good response but crude liver extract was better still. In general, the best response was obtained with 200-300 μg. of vitamin B_{12}. The response to vitamin B_{12} had no correlation, however, with the state of gastric acidity. The authors suggest that in nutritional macrocytic anaemia, folic acid is the primarily deficient factor, whereas vitamin B_{12} is minor and secondary in most cases. Narayanan *et al* (1954) have studied the effect of citrovorum factor therapy in cases of nutritional macrocytic anaemia with r. b. c. less than 2 million/c. mm. and Hb 9.5 g. per cent or below. Twenty four mg. synthetic CF (Leucovorin) intramuscularly caused prompt hæmatological response after a single dose which however, was not maintained beyond two weeks. Two cases of nutritional macrocytic anaemia treated with a single 120 μg. vitamin B_{12} given intramuscularly showed prompt response

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which continued beyond two weeks. Das Gupta *et al* (1956) have also studied the effect of citrovorum factor in nutritional macrocytic anaemia. In their 7 patients 24 to 54 years old with nutritional megaloblastic anaemia and having a dietary history of a poor intake of animal protein, six consecutive daily doses amounting to a total of 18 mg. of leucovorin showed in four cases a good initial response and 36 mg. of leucovorin in another case. One showed fair improvement and one did not respond. The initial response was sustained in only two patients; the others subsequently required folic acid, a high protein diet or crude liver extract.

Oral crystalline penicillin G reported as successful in the treatment of nutritional megaloblastic anaemia in Africans proved a failure in the 6 Indian subjects studied in Bombay by Desai *et al* (1955).

Pernicious anaemia is stated to be essentially a disease of the temperate climate affecting chiefly the white races. Taylor and Chitkara (1940) reported 5 cases of pernicious anaemia in Indians in 1940 and concluded that PA was not rare in Punjab. Das Gupta and Chatterjee (1951) reported 11 years later three cases of PA in Indians in Bengal all of whom had macrocytic anaemia with periodic relapses whenever there was no maintenance liver therapy. The marrow was megaloblastic in all the cases and all of them showed absolute achlorhydria. Sub-acute combined degeneration of the cord was well marked in two but less so in one. In one case neurologic complications developed during folic acid therapy but in this case and in another, liver therapy showed good improvement.

Liver functions in nutritional anaemias :

Das Gupta and Basu (1954) studied the pattern in liver functions in different types of anaemia and in the same patient before and after haematological improvement. The detoxyfying function of the liver as measured by hippuric acid excretion was found to be considerably diminished in all types of anaemia and had definite correlation with the degree of anaemia and was uniformly restored on improvement of anaemia. Evidence of derangement of protein metabolic function of the liver was afforded in macrocytic anaemia.

Pregnancy anaemias :

Some work on the mineral and cholesterol metabolism in anaemias in pregnancy has been reported. In a study on 196 women it has been shown by Mukherjee and Mukerjee (1952) that during normal pregnancy, serum iron decreased steadily from normal non-pregnant values of about 118μg. during the early weeks to a mean value which at term was about

74 μg./100 c.c. In pregnancy complicated either with microcytic or macrocytic anaemia the fall was greater. Blood volume estimations in some subjects showed that there was a slight rise in total serum Fe in the early weeks of pregnancy which was followed by a fall to below normal as pregnancy proceeded. The administration of HCl with meals increased Fe absorption and Serum Fe during pregnancy. Occurrence of diarrhoea had the opposite effect. Serum Fe in both normal and abnormal pregnancy fell rapidly on a diet almost free from Fe. The same authors (1953) also observed an increase in Fe-binding capacity and decrease in Fe-saturation of plasma as pregnancy progressed normally. These effects were exaggerated in Fe deficiency and dimorphic anaemias. In nutritional macrocytic anaemia, Fe-binding capacity was somewhat less than and Fe-saturation was about the same as in normal pregnancy. In normal pregnant women, administration of Fe caused little change in total Fe-binding capacity and an increase in Fe-saturation.

Treatment of anaemias of pregnancy :

In 18 cases of pregnancy anaemia showing megaloblastic bone marrow, free HCl in gastric juice and the absence of sub-acute combined degeneration. Bhende and Kothari (1950) found that crude liver extract, refined liver extract and folic acid were all equally effective even when there were complicating infections.

Chaudhuri (1951) studied the efficacy of vitamin B_{12} therapy in megaloblastic anaemia of pregnancy and tropical nutritional macrocytic anaemia. Pregnant and non-pregnant women all of whom had megaloblastic anaemia responded to treatment with vitamin B_{12} in daily doses varying from 2 to 11 μg. for periods of from 12 to 54 days. The dose required here was larger than the effective dose in the treatment of idiopathic pernicious anaemia of equal severity. All the patients had been on low caloric diets with inadequate protein intake and on admission, the serum protein values (4·9 g./100 c.c.), particularly those of albumin were low in most of them. Most of the patients had oedema of the feet. With rest, improved food and vitamin B_{12}, the serum protein level improved but did not become normal.

Das Gupta *et al* (1953) also studied vitamin B_{12} therapy in 21 patients with macrocytic anaemia during pregnancy or the puerperium, 13 of them with megaloblastic marrow and 8 with normoblastic marrow and found that there was a poor response or none, to small oral doses of vitamin B_{12} but in the majority there was a good initial response associated with a suboptimum reticulocyte crisis after parenteral treatment irrespective of the state of the marrow. The initial response was, however, not maintained but the patients later responded to folic acid or a crude liver extract.

Mukherjee (1951) treated iron-deficiency anaemias with intravenous iron and also dimorphic anaemias. In the latter case haemoglobin level increased initially and then became stationary. At this stage treatment with folic acid or vitamin B_{12} brought about further improvement.

Das Gupta (1954) in a critical review on anaemias in pregnancy has listed many preventive measures that should be taken in India and has recommended some future lines of work.

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DEFICIENCY OF VITAMINS

Vitamin A :

Someswara Rao *et al* (1953) investigated an outbreak of night blindness in a Harijan village 16 miles west of Madras. A nearby village where no case was reported was also studied for comparison. A higher incidence of anaemia in the village where cases of night blindness occurred was observed. Impairment of capacity for dark adaptation was associated with the severity of anaemia. In patients with impaired dark adaptation, an improved diet by itself resulted in an improvement in dark adaptation in 73 per cent of the treated cases, whereas a daily supplement of 130 to 260 mg. Fe or 12,000 I. U. vitamin A raised the improvement rate to 83 per cent and 100 per cent respectively. McLaren (1956) studied 36 cases of keratomalacia, all except 2 in children below 6 years of age, seen during the years 1950 to 1954 in the Phulbani and Ganjam districts of Orissa inhabited by two racially different peoples, the Oriyas and Khonds. There was no marked dietary differences between the Khond tribals and the Oriyas, both having vitamin A deficient diets. Although 5 or 6 Khond children were seen for all medical conditions for every one Oriya, Keratomalacia occurred only in 4 Khonds and the rest of the cases were in Oriyas. The authors suggest that successive pregnancies more rapid than in Khond, might be one of the possible reasons for greater malnutrition in Oriya children than in Khonds. Sen Gupta *et al* (1952) reported that in 136 patients with Kala-azar, signs and symptoms of deficiencies of vitamin A, vitamin B complex and vitamin C, were significantly more frequent than in 40 control subjects consuming more or less the same type of diet. The vitamin A deficiency the author suggested, was due to liver disease. Lahiri and Scandrett (1954) claimed that the results of estimations of blood vitamin A in normal healthy subjects and in patients suffering from skin diseases, provided a basis for relating the lesions in ichthyosis and nummular eczema to vitamin A deficiency.

Dhanda (1956) reported that the electro retinograms obtained in 16 eyes of adults with different grades of xerosis some of which had persisted for years without the development of night blindness, were normal or nearly normal; the concentration of vitamin A in blood serum also remained normal. The author suggested that xerosis was probably not a direct manifestation of vitamin A deficiency. Also in a study of 60 eyes, it was found that the electroretinogram was completely extinguished when the patient became night blind and could be restored by treatment with vitamin A.

Ramalingaswami *et al* (1955) produced xerophthalmia in 2 out of 3 young growing monkeys given a vitamin A deficient diet in a period of 3 to 4 months. Bitot's Spots were seen and the eye-lids were swollen; plasma vitamin A was much reduced. Measurement of the capacity for dark adaptation by threatening the animal silently with a white stick in a dark room, showed responses from the two monkeys with low plasma vitamin A only after from 35 to 45 minutes in the dark, whereas the third animal and two other animals given vitamin A responded after from 4 to 6 minutes. By the end of the fourth month, both the animals with low plasma vitamin A had developed keratomalacia in one eye. Histological studies of the affected eye showed degeneration of the pigment epithelium of the retina and atrophy of the rods and cones. Descemet's endothelium was degenerated. In one animal, only the retina was damaged without involvement of the cornea which suggested that in man, the incidence of damage to the retina through chronic vitamin A deficiency might be much greater than could be assumed from the incidence of keratomalacia.

B vitamins :

Pal (1952) reported a condition of the eyes with lachrymation, slight mucoid discharge and photophobia with a swollen, dull and red conjunctiva as an ocular manifestation of aribioflavinosis found in 5 children and 2 young adults, out of 300 subjects with riboflavin deficiency seen in two years. Banerjee (1952) described a case of pellagra in a widow, aged 40, admitted to hospital with characteristic dermatitis, loss of appetite, inflamed smooth tongue and angular stomatitis, but without diarrhoea or mental disturbance. Her usual diet consisted of rice, dhal and leafy vegetables, no meat and rarely milk. The signs disappeared on a liberal diet including milk or casein with 100 mg. nicotinamide, yeast, HCl and peptone mixture. It was concluded that the patient was suffering from multiple deficiencies including those of nicotinic acid, riboflavin and possibly pyridoxine.

Vitamin C and vitamin D :

Singh (1954) considered scurvy to be the commonest deficiency disease among children in the Punjab. During a period of 5 years 16 cases of infantile scurvy and 4 of rickets were encountered. The children with scurvy were usually between 1 and 2 years of age and belonged to the poor economic group and were weaned on to cow's milk when 4 or 5 months old. Of 600 children attending the Pediatric ward of the K.E.M. Hospital, Bombay, 38 were clinically and radiologically diagnosed by Chitre *et al* (1954) as suffering from rickets; 19 of them were

below 1 year and 2 cases were above 8 years, showing that late rickets also was seen in India. In a majority of cases, serum calcium and inorganic phosphorus were nearer normal values.

Someswara Rao *et al* (1951) examined more than 3,000 persons of various age groups and of various economic status and professions for circumcorneal pigmentation (C.C.P.) and reported that there was no association between nutritional deficiency signs and C.C.P. The authors concluded that vitamin A and riboflavin might indirectly contribute by causing a chronic circumcorneal hyperaemia. The intensity of C.C.P. was, however, found to be directly associated with skin colour; its frequency and severity was greater in dark skinned persons than in fairer ones.

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PROTEIN MALNUTRITION

Nutritional Oedema :

Considerable attention has been devoted by many workers to a detailed study of the various aspects of this deficiency state in adults, the incidence of which is of a high order in the poorest sections of the community. Gopalan *et al.* (1952) described the clinical and biochemical features in 39 cases of nutritional oedema which they treated in Coonoor. They found reduced plasma proteins, due mainly to a fall in albumin. Often, the globulin was found elevated, Plasma Na and plasma chlorides were not elevated. Fasting blood sugar level was low. Alimentry absorption of glucose was delayed. The authors have also described the histological changes in the various organs from biopsy and autopsy specimens. Gopalan and Venkatachalam (1952) have put forward a hypothesis for pathogenesis of nutritional oedema and have suggested that calorie and protein deficiency lead to an increase of antidiuretic hormone in circulation which prevents the normal excretory response to water administration and causes haemodilution resulting in the lowering of intra-vascular osmotic pressure and diffusion of fluid to interstitial tissue spaces. Srikantia *et al.* (1953) reported that the serum Na was at the lower level of normal range in oedema and fell lower still when oedema cleared ; chlorides were below normal both before and after treatment and serum K which initially was at the upper level of normal range, showed a slight fall after disappearance of oedema. The authors concluded that retention of Na was not the cause of nutritional oedema. Gopalan *et al* (1953) studied the body composition in nutritional oedema and found that in oedema, absolute plasma volume values were low ; extracellular fluid, and total body water showed an increase and the cellular solids constituted an abnormally small proportion of the body weight. After high protein therapy, there was a reduction in extracellular fluid both absolutely and relative to body weight, a reduction in total body water and an absolute increase in cellular solids. The clinical grade of oedema was roughly proportional to the product of the amounts of body solids/kg. and extracellular fluid/kg. and intracellular fluid/kg. body weight. Evidence of excessive extra as well as intracellular hydration in oedema was found. Venkatachalam *et al.* (1954) observed that in adult nutritional oedema, the B.M.R. values expressed as per m^2 body surface were low and improved on successful treatment. The values expressed as oxygen consumed per minute/kg. cell solids were, however, not different before and after treatment and hence the authors concluded that in nutritional oedema there is no qualitative reduction in energy metabolism and that the effect found in the B.M.R. values as expressed

normally is entirely due to a reduction in cell solids. The same authors (1955) carried out simultaneous body composition and N balance studies in 8 cases of undernutrition and found that changes in body weight gave a misleading picture of the actual gain in tissues during nutritional rehabilitation. Gopalan *et al.* (1955) reported the changes as found by ECG in the cardiac rate, the rhythm, interval and amplitude in cases of nutritional oedema and nutritional oedema syndrome brought about by the disappearance of oedema following high protein therapy. The cardiac rates which were low in nutritional oedema cases on admission, appreciably rose after treatment, the cycle length was diminished and the amplitude of all deflections increased.

Mukherjee and Warner (1953) reported that in six cases of nutritional oedema which they had investigated, 30 per cent of the fat intake was excreted in stools as against 4 per cent in normals. On treatment with 50 g. amino acid mixture intravenously, total plasma proteins increased SCN space decreased and fat excretion dropped to 6 per cent. Two weeks later during which no amino acid infusion was given, in spite of a high protein diet steatorrhoea relapsed and plasma protein decreased. The same authors (1954) reported that in nutritional oedema cases, there was a linear relationship between plasma amylase activity and serum albumin only when the latter was below 2·5 g/100 ml. ; the correlation of amylase with total protein was not statistically significant; lipase activity was related neither to albumin nor to total protein.

Mazumdar and Mukherji (1951) described three cases of nutritional oedema associated with hypoglycaemic coma. The authors suggested that such signs might be due to deficiency of blood amino acids for conversion to glucose, deficiency of glutamine, deficiency of glutamic acid, malabsorption of sugar, or insufficient carbohydrate intake.

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KWASHIORKOR

The occurrence of a syndrome of malnutrition in infancy and early childhood, characterised by oedema, dermatitis, diarrhoea and fatty liver, described by various workers abroad by such terms as "Kwashiorkor", "malignant malnutrition", and "infantile pellagra", and reported from Coonoor, South India, by Ramalingaswami, Menon and Venkatachalam, in 1948 has been the subject of continued study by many workers chiefly at the Nutrition Research Laboratories, Coonoor. Gopalan and Patwardhan (1951) and Venkatachalam *et al* (1952) reported some major observations on the syndrome which the former authors named as the nutritional oedema syndrome. Chaudhuri (1952) reported 488 cases of "malignant malnutrition" for the period 1947-51, constituting 17 per cent of admissions in the author's wards in Calcutta and with a death rate of 43 per cent. Achar and Benjamin (1953) studied 150 cases in Madras and found skin changes in 56·6 per cent of the cases and palpable liver in 60·5 per cent. Comparatively little of hair changes was found; anaemia was a constant feature and associated vitamin deficiencies were found in large proportion of the cases. On the basis of a study of 207 cases over a period of 4 years, Venkatachalam *et al* (1954) have described in detail the epidemiological, clinical and other features of the syndrome. The peak period of seasonal incidence in Coonoor was in April and May, the "fly season", when the incidence of infective diarrhoea is also at its maximum. Feeding with reconstituted skimmed milk was the mainstay of the treatment. The mortality rate was about 10 per cent. Relapses occurred in 8·5 per cent of the patients which were not related to the intensity of the previous attacks.

From a comparative study of the birth weights of "well-to-do" children, poor class children which later on developed kwashiorkor and those which did not, Varkki *et al* (1955) concluded that the nutritional status of the mother would affect the birth weight of her infant, but would not predispose it to develop nutritional oedema syndrome.

Srinivasan and Ramanathan (1954) estimated the methionine, cystine and protein content of breast milk of apparently healthy Indian labour class women in the Nilgiris and found no significant difference in the values of the milk of mothers in whose progeny no case of kwashiorkor occurred and of those whose children suffered from kwashiorkor. Ramanathan (1955) observed that in children showing nutritional oedema syndrome, the serum values for total protein, albumin, globulin, urea, NPN, total and ester cholesterol were lower than in healthy children and the values, excepting for γ-globulin which was not affected by the syndrome, rose during treatment with a high protein diet and at

discharge were nearly normal. Ramalingaswami *et al* (1952) reported that liver cholesterol in biopsy material from some case of kwashiorkor and adult nutritional oedema was found to be significantly higher than the normal values. There was, however, no correlation between the degree of fatty infiltration and the cholesterol content.

Srinivasan and Patwardhan (1952) found that in kwashiorkor the activity of the enzymes, esterase and lipase in plasma, was lowered and the activity of both returned to normal on treatment with skim milk. The authors also noticed that lowering of plasma esterase showed some correlation with increasing fatty infiltration of liver. Mukherjee and Sarkar (1955) reported from a study of liver biopsy specimens, that in nutritional oedema and kwashiorkor, the liver enzymes catalase and alkaline phosphatase were raised and choline esterase reduced.

Ramachandran *et al* (1956) found that the 24-hour urinary excretion of 17-ketosteroids in adult cases of nutritional oedema and cases of nutritional oedema syndrome in children, were lower than in corresponding normals. After high protein therapy, the 17-ketosteroids excretion increased in all patients, but normal values were reached earlier in children than in adults.

Mukherjee (1955) has reported an excretion of fat as high as 130 to 257 per cent of the total intake in his series of kwashiorkor cases. On treatment with skimmed milk, fat balance became positive in 3 to 6 days. Similarly the N balance also changed from negative to positive.

Mehta *et al* (1956) reporting steatorrhoea in 6 children with nutritional oedema syndrome observed that when the dietary fat was low more of it was excreted than was ingested and increased intakes of fat or glucose led to greater excretion of fat. Groundnut oil increased faecal fat to a greater extent than did an equal amount of butter fat. They considered the faecal fat in kwashiorkor subjects to be partly endogenous and partly exogenous in origin. Wheat did not aggravate steatorrhoea and folic acid had no beneficial effect. Mukherjee and Sarkar (1956) reported that in kwashiorkor 60 to 80 per cent of the ingested fat was excreted in the first 24 hours, and some times even more than the ingested amount and in marasmus 9 to 20 per cent. After clinical recovery from kwashiorkor only 12 to 18 per cent of ingested fat was found to be excreted.

The pathological changes in kwashiorkor have also been studied in detail. Gopalan and Patwardhan (1951) and Achar and Benjamin (1953) reported severe atrophy and degeneration of the acini of pancreas in kwashiorkor. Sriramachari and Ramalingaswami (1953) and Ramalingaswami *et al* (1954) reported in great detail, the structural changes

taking place in kwashiorkor. The latter authors concluded that the liver injury in kwashiorkor is to a great extent due to protein deficiency alone and consists of a marked reduction in cytoplasmic basophilic particles, presumably ribonucleoproteins and an increase in alkaline phosphatase activity.

Ramanathan *et al* (1955) chose 100 children from records of previously cured cases, for a follow-up study and found that 20 of them could not be traced, 40 had had no relapse and 11 had been treated successfully for recurrence on one or more occasions and 29 had died, 19 of them as the result of unattended relapses after an interval of from 6 months to about 5 years. In all, 37·5 per cent of cases that could be traced, had relapses from which they had either recovered or died. 50 per cent of the surviving children exhibited specific signs of nutritional deficiency. It was interesting to note that in spite of no improvement in diet and environmental conditions, 50 per cent of the cases had shown no relapses and were apparently normal.

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LIVER INJURY

Experimental :

Govindan and Radhakrishna Rao (1953) reported that under the same dietary conditions, the hepatic fatty infiltration produced in animals during summer was higher than that produced in winter. The lower liver fat in animals in winter, the authors suggested, might be due to (i) greater utilisation of dietaty fat for calorie requirements at low temperatures, or (ii) higher requirements for choline and other B vitamins at higher temperatures resulting in a more acute deficiency of the vitamins, especially choline. In their detailed study of nutritional factors in toxic liver injury, Patwardhan *et al* (1953 and 1954) observed severe reduction in the cytoplasmic basophilia and alkaline phosphatase reaction in centrilobular zones associated with marked increase in the alkaline phosphatase reaction in the surrounding periportal zones of the hepatic lesion both in the prenecrotic and necrotic stages. In liver, the activity of succinic dehydrogenase and transaminase decreased and that of alkaline and acid phosphatase and glutamic dehydrogenase increased; the increase in alkaline phosphatase was the most conspicuous change observed. After a single injection of carbon tetrachloride total liver N, continued to decrease upto 120 hours studied and marked reduction in liver glutathione content was observed 6 hours after CCl^4; methionine content was found unaffected. The authors also reported that methionine and glutathione had no clearcut effect on the necrotic or prenecrotic phases of CCl^4 action, although not infrequently the onset of necrosis was delayed and the extent of the damage less under their influence. The main effect was, however, seen in the regeneration phase, which was accelerated by the administration of methionine or glutathione.

Cirrhosis :

Veliath and Srinivasachar (1957) studied 121 cases of portal cirrhosis, treated in the three main hospitals in the then Madras province of South India, which later came up for post mortem examination and found that 57 cases showed a histological pattern of fatty infiltration with varying degrees of fibrosis, which the authors believed, was due to dietary deficiency of lipotropic factors; 24 cases of cirrhosis followed attacks of jaundice and 40 cases showed postnecrotic cirrhosis in whom history of jaundice was not available and hence the aetiology was unknown. Alcohol was found to play no significant role in the aetiology of cirrhosis in Madras Province. Govindan and Radhakrishna Rao (1952) found that the mean serum choline contents of 17 normal

subjects and of 31 adults and 7 infants with liver cirrhosis, did not differ significantly. On a study of hepatic structure and functions with liver biopsy material from 88 cases of nutritional oedema and 45 cases of liver cirrhosis with oedema and ascites, Sriramachari and Ananthachari (1954) found that vacuolar degeneration and fatty infiltration of liver were milder in precirrhotic stages of hepatic damage; mesenchymal reactions with fibrosis were seen to a limited extent in malnutrition but regularly in cirrhosis. A reduction in serum albumin and a large increase in γ-globulin were seen in both malnutrition and cirrhosis. In both groups, fibrotic changes were associated with rises in γ-globulin and strongly positive flocculation tests.

Infantile cirrhosis of the liver :

Misra (1950) reported 97 cases of infantile cirrhosis from Uttar Pradesh, Mukerji (1952) 200 cases from Indore, and Srivastava and Aikat (1954) 45 cases. Maximum incidence of the disease was in the age group 1-2 years. Mukerji, (1952) and Srivastava and Aikat (1954) 88 per cent and more of the cases occurred in children below 3 years Misra (1950) and Mukerji (1952). The disease affected predominantly the male child Mukerji, (1952) and Srivastava and Aikat (1954). A very large majority of the cases were from the lower middle, upper middle and middle classes Misra (1950) and Mukerji (1952). In Misra's series 94 out of 97 patients were Hindus. In Mukerji's 200 cases, 178 cases were from Jain and Hindu vegetarian families and only 14 from Hindu non-vegetarian families. 53·6 per cent of the cases had history among siblings (Misra, 1950). Maximum incidence was in the first child and next in incidence was the third child (Mukerji 1952). Misra (1950) described three stages of the disease; all in first stage, and 39 out of 44 cases in second stage, were cured and 19 in the third stage died. Mukerji (1952) has described in detail frequency of various clinical signs in his 200 cases.

Bhende and Deoras (1954) examined histopathologically 9 autopsy liver specimens and found that connective tissue showed inflammatory cells in every specimen, plenty in earlier and less in later stages. Necrosis of liver cells was found to be the primary and the most constant change at all stages, showing that the process was kept going by continued attacks. From a study of over 250 cases during a period of 4 years, Achar and Chacko (1954) reported that widespread necrobiotic changes not found in infective hepatitis or kwashiorkor sections of liver, were a common feature in infantile cirrhosis. There was annular fibrosis and pseudolobulation was a feature even in early cases; bile duct proliferation was found only in the late cases. Srivastava and Aikat (1954) reported

that in addition to hypoproteinemia and hypoalbuminemia, liver function tests revealed gross abnormality with thymol turbidity and cephalin cholesterol flocculation tests. Serum bilirubin and urinary urobilinogen increased; serum alkaline phosphatase was normal. There was diffuse hepatic fibrosis. The authors concluded that Rh incompatibility was not the cause, and that an infective virus might be one of the aetiological factors.

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PHRYNODERMA

From a study of the cutaneous lesions resembling phrynoderma, produced in rats in vitamin A and in essential fatty acid deficiency, Kolah and Radhakrishna Rao (1953) confirmed the role of essential fatty acids in the maintenance of healthy condition of the skin as reported by previous workers (Gopalan, C, Indian Med. Gaz., **82**, 16, 1947; Menon, P. S. Tulpule, P. G. and Patwardhan, V. N., Indian J. Med. Res., **38**, 173, 1950). Rajagopal and Chowdhury (1952) reported that total lipids, cholesterol and unsaturated fatty acids were lower in the serum of phrynoderma patients than in normals and improved on treatment with linseed oil, confirming the findings of Menon *et al* (*loc. cit*).

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GOITRE

Goitre, deaf-mutism and cretinism are prevalent in the northern regions of India from the north-west frontier covering Kashmir, Nepal, Bihar and Assam upto Burma. Ramalingaswami (1953) discussing the problem of goitre prevention in India observed that, among other things, bacterial pollution of drinking waters, excessive intake of calcium, faulty and unbalanced diet all had a role to play in the etiology of the disease. The author pointed out the inherent difficulties in iodising the coarse crystalline salt obtained by solar evaporation of brine and in stabilising its iodine content under the hot and humid atmosphere in India. The adding of salt during cooking as is the general practice in India also entailed loss of iodine. Sen Gupta and Swarup (1956) reported that out of 8,204 children examined according to Ryle's classification of thyroid enlargement in the Sub-Himalayan region of West Bengal, 67 per cent had goitre. 59·8 per cent of the children were suffering from tuberculous lymphdenitis. 49 per cent had both tuberculous infection and goitre. The authors suggested that hypothyroidism might increase the susceptibility of goitrous subjects to tuberculous infection.

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EPIDEMIC DROPSY

Lal (1951) studied eight outbreaks of epidemic dropsy in Bihar and found that members of the middle-class families only were affected and that the incidence of the disease was comparatively more in Bengalis than in other communities and in males than in females; no case in children under 8 years was reported. The cause in all cases was the consumption of mustard oil adulterated with argemone oil. Mitra (1951) reported that argemone oil, even after freeing from its alkaloids, produced oedema of the feet in guinea-pigs. Chakravarti and Chaudhuri (1951) and Lal (1954) have reported production of epidemic dropsy in monkeys given argemone oil by stomach tube or by intramuscular injection. The toxic signs noted included apathy, failure of appetite, oedema, erythema and death. Haemorrhagic points were seen in lungs and digestive tract *post mortem*. There was some evidence that argemone oil upto 3 per cent in mustard oil was not lethal.

Bhatia and Malaviya (1951) produced an epidemic-dropsy-like syndrome in volunteers from Lucknow jail by daily administration of 1 oz. of mustard oil adulterated with 10 per cent argemone oil for 28 days. A control subject receiving pure mustard oil did not develop the characteristic symptoms of general malaise, alimentary disturbances and oedema. Changes in the cardiovascular system, visual fields or the tension of the eye-balls were not found.

Chakravarty *et al* (1953) studied the body fluid changes in 12 patients with epidemic dropsy and observed that compared to normals, the values for plasma and blood volumes, available thiocyanate space and interstitial fluid were higher in them and the value for red blood cell mass was lower. The anaemia accompanying the disease, the authors concluded, was not the result of blood dilution but that of reduction in the number of circulating red cells.

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LATHYRISM

Roy (1951) reported a study of over 8,000 cases during an outbreak of lathyrism in Madhya Pradesh in 1945 and found that the poorest classes who were paid their wages mostly with a mixture of wheat, Bengal gram and Khesari (*Lathyrus sativus*) gram were the ones affected. Mainly those who were previously consuming fairly large quantities of *Lathyrus sativus* in the form of *chapatis* were affected whereas persons who were consuming only small amounts in the form of cooked *dal* were free from the disease. Rudra (1952) reported that "Khesari" dal contained an abnormally high amount of selenium compared to other pulses and this, he maintained, was the toxic factor in *L. Sativus*. He also reported that injections of selenium into monkeys produced typical signs of lathyrism. Selenium, the author suggested interfered with the utilisation of sulphur and thus with the metabolism of thiamine, biotin and methionine. However, Seshadri *et al* (1955) could not find any significant difference in the selenium content of khesari and other commonly consumed pulses and could not confirm Rudra's finding.

Rudra *et al* (1952) reported that in 2 of the 5 patients with lathyrism, who were injected 1 to 2 g. methionine intravenously for several days, Babinski's sign disappeared and one of the patients regained 75 per cent normal gait.

Bhargava (1956) failed to produce lathyrism in pigeons by feeding for a period of 12 weeks a diet chiefly of dry, germinating or boiled seeds of a pure strain of *Lathyrus Sativus*.

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FLUOROSIS

Investigations on the removal of fluoride from water have been carried out by a few workers (Venkataramanan and Krishnaswamy 1951), (Narayana Rao and Venkateswarlu 1951), (Venkateswarlu and Rama nathan 1952), (Venkateswarlu and Narayana Rao 1953 and 1954)

In an examination of over 1,000 children belonging to 12 different villages whose waters contained from 0·3 to 1·6 p.p.m. of fluorine, Venkateswarlu *et al* (1952) observed that the incidence of mottled enamel was directly proportional to the fluorine content of the water supplies; the incidence of caries was inversely related to the fluorine in water. The incidence of mottled enamel and caries, however, varied highly in communities exposed to nearly the same amount of fluorine through drinking water. In such communities, the incidence of caries was found to vary inversely as the Dental Fluorosis Index. The authors suggested that the optimal (sub-mottling) concentration of fluorine in drinking water for mass caries control should be from 0·5 to 0·8 p.p.m. Venkateswarlu and Narayan Rao (1953) reported analysis of over 170 samples of surface, spring and well waters from the Visakhapatnam district and found the fluorine content varying from a trace to 11 p.p.m. However, most of the samples were generally between 0·5 and 2 p.p.m. Some places showed wide local variations attributed to the presence of F-bearing apatite rocks. Satyanarayana Murthi *et al* (1953) examined the population of the district of Visakhapatnam and the suburban areas for "stiff back". One adult male with advanced fluorosis and two relative of his with stiff-backs were found, all of whom were consuming water, with a fluorine content of about 11 p.p.m. The incidence of stiff-back was not high as was apprehended from the high fluorine content of the water. "Stiff-back" was unknown in areas where the water contained fluorine in amounts less than 3·8 p.p.m.

Wadhwani (1954) X-rayed the skeletons of 9 patients having signs of fluorosis. The X-ray pictures taken after daily supplement to each of 100 mg. vitamin C for 6 weeks showed decrease in the size of exostoses; all patients reported less pain, reduced tingling sensation and improved limb movement.

Wadhwani (1953) reported that monkeys receiving 10 mg. NaF/kg. body weight daily for 6 months and 20 mg. vitamin C daily did not develop fluorosis, whereas the mortality in those receiving the same amount of fluorine and a vitamin C deficient diet was higher than in controls; the stiffness of limbs and restriction of movement were relieved in surviving animals when 20 mg. vitamin C was given daily. In animals

with severe fluorosis, mere discontinuing of F did not improve the condition, whereas in addition a daily supplement of 20 mg. vitamin C effected great improvement with reduction or disappearance of exostoses. Wadhwani (1952) also observed that in rats and monkeys, chronic fluorosis of the same type caused a reduction in the vitamin C content of the soft tissues and a rise in the percentage of iodine in the thyroid gland both being more marked in the monkeys than in rats.

Venkateswarlu and Narayan Rao (1954) reported that in guineapigs fed fluoride and maintained on different levels of vitamin C, no difference could be found in the amount of fluorine stored in the skeleton. The authors, hence concluded that vitamin C status of nutrition, did not affect the accumulation of fluorine in skeleton.

Wadhwani (1954 and 1955) studied the effect of fluorine on the composition of bones in rats and monkeys. In rats fluorine decreased the N content of dry fat-free bone and increased the sodium and carbonate content. Higher dosage of fluorine, besides exaggerating these effects also increased the Ca and in most bones also the P content. Monkeys fed NaF at a dosage of 10 mg./kg. body wt. daily for 27 weeks showed on being X-rayed, distinct thickening of the periosteum and formation of exostoses. Exostoses were almost absent from bones after the administration of 20 mg. vitamin C daily for 12 weeks to monkeys with severe bone fluorosis.

Wadhwani and Ramaswamy (1953) have reported the histological changes in the various tissues of fluoride-fed albino rats and monkeys.

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